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BIOREMEDIATION METHODS ASSISTED WITH HUMIC ACID FOR THE TREATMENT OF OIL-CONTAMINATED DRILL CUTTINGS

MÉTODOS DE BIORREMEDIACIÓN ASISTIDOS CON ÁCIDO HÚMICO PARA EL TRATAMIENTO DE RECORTES DE PERFORACIÓN CONTAMINADOS CON ACEITE

M.L. Interiano-López[1], V.A. Ramírez-Coutiño[2], L.A. Godinez-Tovar[1], E. Zamudio-Pérez[1], F.J. Rodríguez-Valadez[1]*

[1]*Centro de Investigación y Desarrollo Tecnológico en Electroquímica S.C. Parque Tecnológico Querétaro Sanfandila, Pedro Escobedo. Querétaro. C.P 76703, México.*
[2]*Universidad Tecnológica de México, UNITEC-México, Campus Querétaro, Av. 5 de Febrero No. 1412, San Pablo, C.P. 76130, México*

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Abstract

In this study it is reported the treatment of oil-based drill cuttings (OBDC) by the analysis of performance 8 biopiles placed within reactors. Biopiles were prepared with a mixture of drill cutting, bulking agents, microbial consortium, inorganic nutrients and humic acid (HA) solutions at 500, 1,000, 1,500, 2,000, 2,500 and 3,000 mg/L. Experimental follow-up was done registering temperature, CO_2 generation and pH. After 8 weeks, oil removal was evaluated using total petroleum hydrocarbons (TPH) soxhlet extraction, the highest value was 72% removal of TPH obtained with the biopile of 1,500 mg/L of HA. Also, this biopile showed higher temperatures, higher CO_2 generation and the lowest pH, indicating a more active biological system compared with other concentrations. The obtained results show that the addition of HA significantly improves the performance of biopiles, indicating that the combination of technologies employed with addition of HA are effective for the treatment of oil-based drill cuttings.

Keywords: Oil-based drill cuttings, total petroleum hydrocarbons, bioremediation, bioaugmentation, biostimulation, humic acids.

Resumen

En este estudio se informa el tratamiento de recortes de perforación base aceite (OBDC por sus siglas en inglés) mediante el análisis del desempeño de 8 biopilas dentro de reactores. Las biopilas se prepararon con una mezcla de recorte de perforación, agentes volumétricos, consorcio microbiano, nutrientes inorgánicos y soluciones de ácido húmico (HA) a 500, 1,000, 1,500, 2,000, 2,500 y 3,000 mg/L. El seguimiento experimental se realizó registrando la temperatura, la generación de CO_2 y el pH. Después de 8 semanas, la extracción de aceite se evaluó utilizando extracción soxhlet de los hidrocarburos totales del petróleo (TPH por sus siglas en inglés). La mayor remoción de TPH fue del 72% obtenida con la biopila de 1,500 mg/L de HA. Además, esta biopila mostró temperaturas más altas, mayor generación de CO_2 y el pH más bajo, lo que indica un sistema biológico más activo en comparación con otras concentraciones. Los resultados obtenidos muestran que la adición de HA mejora significativamente el rendimiento de las biopilas, lo que indica que la combinación de tecnologías empleadas con la adición de HA es eficaz para el tratamiento de los recortes de perforación base aceite.

Palabras clave: Recortes de perforación base-aceite, hidrocarburos totales del petróleo, biorremediación, bioaumentación, bioestimulación, ácidos húmicos.

1 Introduction

Petroleum hydrocarbons, undoubtedly constitute a key element for human activities because they are the main source of energy as well as raw material for the manufacture of many products. Oil exploitation has been done by drilling wells, in which it is required drilling fluids containing oil-based chemicals to lubricate the drill bit. Nevertheless, the drilling processes used for obtaining petroleum produce large amounts of waste (Breuer *et al*., 2004; Leonard S.A. *et al*., 2010; Ma *et al*., 2016).

* *Corresponding author. E-mail*: frodriguez@cideteq.mx
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The mixture of rock fragments and muds generated in the process are called drilling cuttings, these are impregnated with the drilling fluids; this material represents an environmental risk because large volumes are generated, plus there is toxicity associated with the presence of hydrocarbons, drilling fluids (Ayotamuno *et al*., 2009; Ball *et al*., 2012; Bakke *et al*., 2013). Current disposal of these materials includes reinjecting the cuttings to the wells, or transporting the cuttings for treatments like incineration, solidification/stabilization, microwave treatments and supercritical fluid extraction (Marwa and Abir, 2007; Street and Guigard, 2009; Robinson *et al*., 2010; Ball *et al*., 2012). An interesting alternative for treatment of this material can be found in biological processes, because they are environmentally friendly and particularly attractive because of their low cost and relatively simple maintenance (Mirsal, 2008). Composting can act accelerating the biodegradation that naturally occurs in contaminated environments, for the biostimulation it is required the addition of organic matter and nitrogen obtained for wood chips, manure, straw bird droppings, sawdust, grass clippings, maize straw and sweet sorghum bagasse (Ayotamuno *et al*., 2009). This kind of treatment can be developed using static biopiles or in vessel systems like bioreactors (Ouyang *et al*., 2005; Marín *et al*., 2006; Choi end Chang, 2009). If content of native microorganisms is not enough to degrade the pollutants, microbial consortia or specific microbes such as bacteria, fungi or enzymes can be added (bioaugmentation) to increase the rate of degradation (Okparanma *et al*., 2009; Fernández-Luqueño *et al*., 2011; Fuchs *et al*., 2011; Lebeau *et al*., 2011; Agnello *et al*., 2016; Cisneros *et al*., 2016; Ma *et al*., 2016; Martínez and Soto 2017). Some authors have preferred using a consortium rather than a pure microbial culture since the latter have limitations to fully accomplish pollutant degradation. It is reported that biotransforming the toxic compounds only reach to intermediates compounds in the degradation pathway (Fantroussi and Agathos, 2005; Tyagi *et al*., 2011; Derrossi *et al*., 2014). On the other hand, some reports provide evidence that the removal efficiency can be limited by the bioavailability of the contaminants, so the presence of surfactants can be crucial because they act reducing surface tension at air-water interface resulting in an increased solubility and bioavailability of the contaminants (Urum *et al*., 2006; Yan *et al*., 2011; Cerqueira *et al*., 2014). Use of biosurfactants like aescin, lecithin, rhamnolipid, saponin and tannin, takes particular relevance since they are natural substances that do not exert contaminant effects in the environment (Urum and Pekdemir, 2004). Another biosurfactant are HA, these acids have gained greater interest for the application in bioremediation of contaminated environments by persistent organics in soils; HA have an amphiphilic structure which could act increasing solubility of the organic compounds (Perminova *et al*., 2005; Klavins and Purmalis, 2010; Ramírez-Coutiño *et al*., 2013). In a previous work, Conte *et al*. (2005) evaluated hydrocarbons removal in a highly contaminated soil by using the surfactant aided washing process, they applied different synthetic surfactants and HA, their findings have shown that HA allowed to get removals between 80%-90%, this was the first time that it was reported that a natural nontoxic surfactant, such as HA, has the capacity for removing contaminants from a polluted soil in similar amounts to that obtained with synthetic surfactants. The aim of present study is to set up the basis for a biological treatment of oil-field drill cuttings mud, a methodology based on comparison of the composting process aided by a combined bioaugmentation - biostimulation plus HA, and doing the analysis of effectiveness in terms of TPH reduction percentage.

2 Materials and methods

2.1 Materials

All reagents like solvents, chemicals and nutrients used in the analysis of the sample treated and microbial consortium as C_6H_{14}, $Ba(OH)_2$, $FeSO_4 \bullet 7H_2O$, $K_2Cr_2O_7$, H_2SO_4 and H_3PO_4, $Fe_2Cl_3 \bullet 6H_2O$, $CaCl_2$ and $MgSO_4 \bullet 7H_2O$ were of analytical grade and were obtained from J.T. Baker Company. HA used were obtained from compost produced with mixing 30% municipal sewage sludge, 60% grass and 10% wooden shavings with a micellar critical concentration (CMC) of 2,000 mg/L (Ramírez-Coutiño *et al*., 2013). The OBDC used in the experiments were obtained from an installation located in the Veracruz State, Mexico.

2.2 Drilling cuttings characterization

The OBDC sample was dried at room temperature during 4 days. The physico-chemical characterization was carried out determining texture, real density, nitrogen, organic matter (OM), total organic carbon

(TOC), phosphorus, pH, electrical conductivity (EC), moisture and TPH measurements. TPH analyzes were performed based on EPA 9071B, a method which is used to quantify the concentrations of oil and grease in soil, sediments and sludge; it uses n-hexane as solvent with soxhlet extraction, the results of this method are named as TPH. For the TPH quantification analysis, a 15 g sample of drilling cuttings was weighted, wrapped in filter paper, placed in an extraction thimble, and soxhlet extraction was run for 7 h. The total amount of extract containing the compounds of TPH was estimated by weighing the dry residue after evaporating the solvent. Each analysis was done in triplicate and the reported result is the average of the 3 measurements.

2.3 *Microbial consortium*

The microbial consortium was prepared from an OBDC sample as follows: 500 g of the OBDC sample were collocated in a reservoir having 3 L of water, in which 1 mL/L of nutrients solution (solution of phosphate buffer, $Fe_2Cl_3 \bullet 6H_2O$, $CaCl_2$ and $MgSO_4 \bullet 7H_2O$) and oil 0.5 mL/L were added, aeration was applied by using an air pump. Later, the biomass was separated in successive steps until the elimination of inorganic solids. For the inoculum preparation, a column with a capacity of 3 L was used; a microbial culture obtained of drilling cuttings previously separated by centrifugation at 2,500 rpm for 10 min was added in an amount of 9 g (3 g/L). Every week 0.5 mL/L of OBDC oil and 1 mL/L of nutrients solutions were added under aerobic conditions.

2.4 *Experimental procedure*

2.4.1 *Bioreactors set-up and operating conditions*

Cylindrical bioreactors, 0.32 m x 0.20 m height and diameter, respectively, were made of acrylic and provided with three sampling ports; at the middle section it was placed a port to insert a glass thermometer, experimental arrangement is show in Fig. 1. The air supplied to bioreactors was CO_2 cleaned by passing it through three flasks containing $Ba(OH)_2$ 0.125 M. In order to quantify the CO_2 generated by microbial degradation of the hydrocarbons contained in the drilling cuttings, at the output of the bioreactors, another three flasks with $Ba(OH)_2$ 0.125 M were placed.

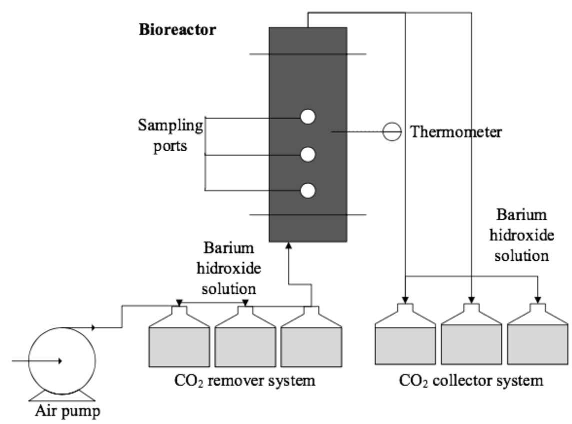

Fig. 1. Schematics of a bioreactor unit.

Table 1. Composition of material in the bioreactors.

Biopile	OBDC (kg)	Bulking agents Grass (kg)	Weeds (kg)	Bioaugmentation Inoculum (mL)	Biostimulation Ratio N:P (mL)	Humic acid (HA) (mg/L)
OCDC	3	-	-	-	-	-
BACOMP	0.75	1.5	0.75	500	500	-
BAECH500	0.75	1.5	0.75	500	500	500
BAECH1000	0.75	1.5	0.75	500	500	1,000
BAECH1500	0.75	1.5	0.75	500	500	1,500
BAECH2000	0.75	1.5	0.75	500	500	2,000
BAECH2500	0.75	1.5	0.75	500	500	2,500
BAECH3000	0.75	1.5	0.75	500	500	3,000

2.4.2 *Bioremediation treatments*

Each experiment bioreactor was charged with a mixture containing OBDC, grass clippings and weeds sticks as bulking agents in a ratio 1:2:1, respectively. Bioreactors were enriched with 500 mL of a microbial consortium containing 1.6 x 109 CFU (colony forming unit) /mL, which was previously adapted to TPH from OBDC. The required nitrogen and phosphorus were added in a ratio proportional to the carbon content in the biopile as follows C:N:P, 100:10:1. To evaluate the HA biosurfactant effect on TPH´s removal, HA solutions ranging between 500 to 3,000 mg/L were added (see Table 1).

2.4.3 *Evaluation of bioreactors performance*

Experiments of TPH removal in bioreactors were monitored over a period of 8 weeks, and TPH concentration was determined by using the gravimetric method with soxhlet extraction equipment as described in section 2.2. Temperature in the bioreactor was monitored through a mercury-in-glass thermometer, and pH was analyzed using a portable pH meter (Thermoscientific brand). The CO_2 generated by microbial respiration due to degradation TPH was trapped in $Ba(OH)_2$ 0.125 M solution and measured by either titration of the residual hydroxide or as inorganic carbon in each system according to the methodology proposed by the OECD Guideline for Testing of Chemicals: 301 B CO_2 Evolution Test, (1992). This was done before, during and after treatment by the eight-bioremediation methods.

3 Results and discussion

3.1 *Chemical and physical analyses*

Table 2 shows the physical and chemical characteristics of OBDC sample used in this study. The sample has a TPH content of 116,372 mg/kg, a value being significantly high therefore it represents a serious pollution problem. This quality criteria agrees with the fact the sample also has a high content of organic matter (6.3%) which is related with hydrocarbons. Soil sample texture was identified as loamy sand, its electrical conductivity of 15.71 μS/cm is in agreement with this classification; soil moisture content was 29%, basic pH (value 9.12).

Table 2. Physicochemical characteristics and TPH present in the OBDC sample.

Parameter	Result
Conductivity	15.71 μS/cm
pH	9.12
Real density	2.35 g/cm^3
Moisture	29%
Texture	Loamy sand
Organic matter	6.30%
Total organic carbon	100,000 mg/kg
Total phosphorus	340 mg/kg
Total nitrogen	509 mg/kg
Total petroleum hydrocarbons (TPH)	116,372 mg/kg

This high pH is determined by the drilling cuttings chemical composition since it is a mixture of clay, quartz, feldspars and carbonates (Ma *et al*., 2016). It was detected that the nitrogen and phosphorus content is low, which may be a limiting factor for contaminants degradation due to lack of nutrients for microorganisms.

3.2 *Bioremediation experiments*

3.2.1 *Temperature profiles*

Temperature is used to monitor the process, because its evolution is related to many of the biological reactions that take place within the bioreactors (Kazemi *et al*., 2016). Temperature of the samples in the bioreactors fluctuated between 21 and 34 ºC during the experimental stage.

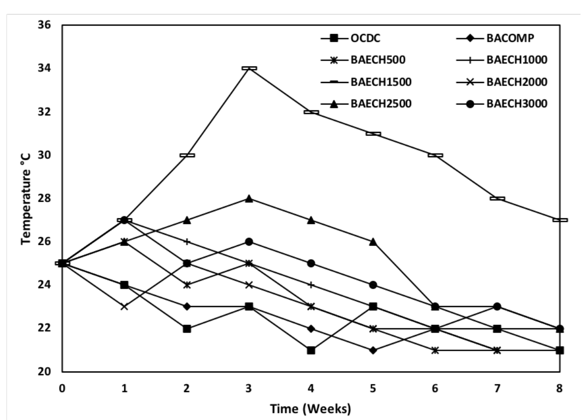

Fig. 2. Temperature profiles in bioreactors.

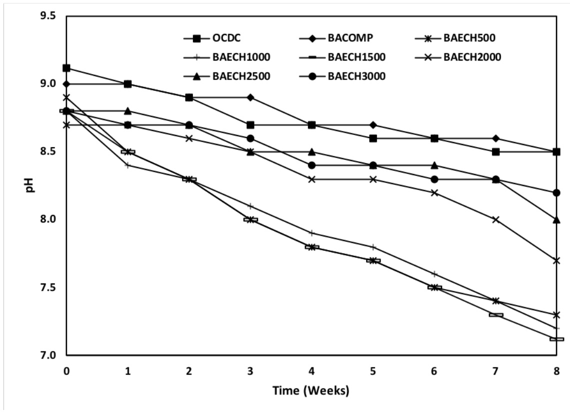

Fig. 3. pH evolution inside the biopiles.

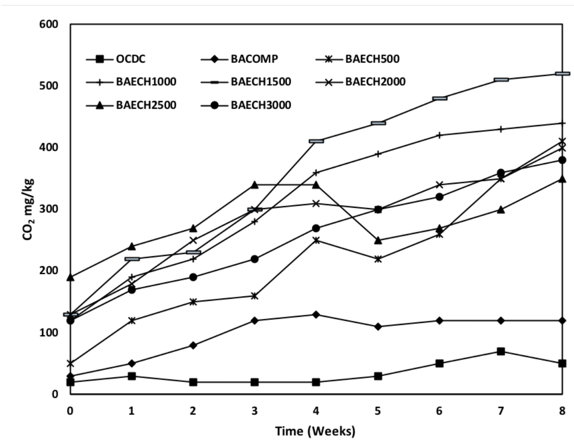

Fig. 4. Cumulative CO_2 production by respirometry in biopiles during 8 weeks of bioremediation.

BAECH1500 and BAECH2500 treatments exhibited an increasing trend during the first 3 weeks and later a decreasing trend; otherwise BAECH1000 and BAECH 2000 exhibited a moderate increasing trend during the first week (Fig. 2). The higher temperatures were detected with BAECH1500 treatments. The increase in temperatures in the first three weeks was due to the high metabolic activity of the microorganisms fact that could be associated with the bioavailability of substrate (TPH) and nutrients. In this particular case, the application of 1,500 mg/L of HA favored the highest temperature in the samples so it can be affirmed that this addition enhanced the biological activity of the system. The subsequent decrease in temperature in the fourth week could be due to the reduction of organic matter susceptible of being degraded by microbial activity (Lee *et al*., 2009).

3.2.2 pH evolution

Observed values of pH in the bioreactors range between 7.1-9.0, with a decreasing trend (Fig. 3), initial values are strongly influenced by the pH of the OBDC sample (pH= 9.12). Initially pH of samples were in the range of 9.1 and 8.7. The largest reduction in the pH value was registered in BAECH1500 sample, reaching a pH value of 7.1. Behavior which can be attributed to the degradation of the hydrocarbons, which may have released acidic intermediates and final products, resulting in a pH lowering of the mixture (Adhikari *et al*., 2009).

3.2.3 Microbial respiration

The respiration rate, measured by carbon dioxide production, was selected as one of the parameter to assess aerobic biological activity because CO_2 is indicative that the organic matter is being degraded (Said-Pullicino *et al*., 2007; Xiao *et al*., 2009). Fig. 4 shows CO_2 evolution for all bioreactors, it is observed that the highest production occurred in the reactors in which HA were added, the microbial respiration was higher than that observed in the control reactors and BACOMP without addition of HA. These results indicate that HA contribute to increase microbial activity, this is probably because the HA dispersed part of the hydrocarbons contained in the drilling cuts, facilitating bioavailability and biodegradation, fact which implies higher release rates of CO_2. Focusing on the reactors where HA was added, the BAECH1500 was the one that produced the greatest amount of CO_2.

3.2.4 TPH Removal in bioreactors with and without HA addition

Changes in TPH content occurring in bioreactors composted with HA were significantly different from the control experiment and BACOMP (Fig. 5). In the first three weeks, it is observed a decrease in TPH levels between 40-50% in the reactors with HA and between 10% and 15% in the control and BACOMP respectively. Analyzing the group of bioreactors with HA, it is observed that final TPH removal in the BAECH1500 was 72% at the end of 8 weeks, this response is in agreement with the largest heat generation, CO_2 production and pH changes as was discussed in sections 3.2.1 to 3.2.3.

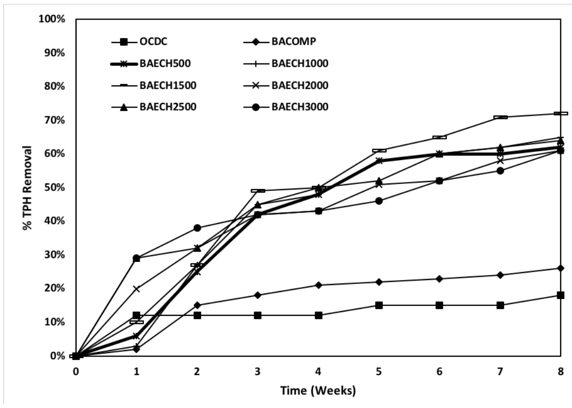

Fig. 5. Removal of TPH using bioremediation with and without addition of HA.

The observed highest degradation in the BAECH1500 system could be explained by the presence of the HA that could be acting as a surfactant enhancing the dispersion of TPH (Yanto *et al*., 2017), increasing the activity of the biological system that produce higher temperatures and so far larger CO_2 production.

Analysis of Fig. 5 indicates that the best results of contaminant removal were obtained with a concentration of 1,500 mg/L HA, which is below the critical micelle concentration. This phenomenon can be explained because not necessarily HA need to be at the critical micelle concentration to solubilize nonpolar contaminants as reported by von Wandruszka, (2000) who found that in solutions at lower concentration than critical micelle concentration HA can built aggregates similar to micelles, which are known as a pseudomicelles. Due to its length and flexibility, the humic polymer chain can be twisted so that the *hydrophilic groups* (carboxy and hydroxyl groups) are directed outside the molecule while the *hydrophobic groups* (for example, hydrocarbons) were maintained inside the aggregate, these act facilitating the solubilization of different nonpolar solutes.

It should be noted that in the first three weeks the highest percentages of removal were obtained in HA treatments. The largest increases at concentrations of BAECH2000 and BAECH3000 in the first week were generated and then decreased. In the case of BAECH1500 the increase in the first week is smaller, however the TPH / time change ratio increases with time which results in a greater removal in the BAECH1500 treatment.

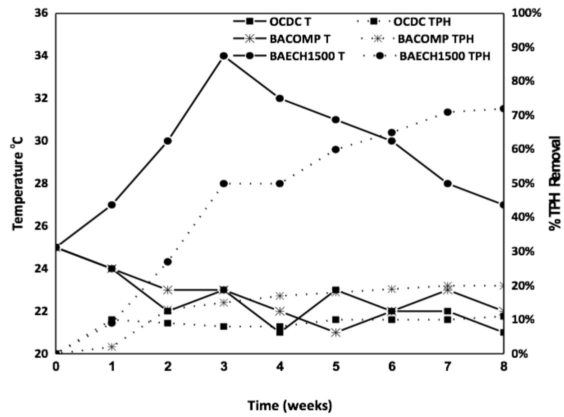

Fig. 6. Temperature (T continuous line) and Removal of TPH (TPH dotted line) in OCDC, BACOMP and BAECH1500.

3.2.5 Temperature profile and TPH removal

One of the main parameters that indicate the degree of removal of organic matter in composting processes is temperature. In order to relate the high temperatures with the highest percentages of removal of contaminants, the temperatures and percentages of TPH removals that were obtained during the 8 weeks of treatment in the OCDC, BACOMP and BAECH1500 samples are shown in Fig. 6.

It can be noted that all the treatments started with a temperature of 25 ºC, in the case of the OCDC and BACOMP bioreactors, the temperatures decreased with respect to the passage of time reaching at the end of the process 21 ºC and 22 ºC respectively, also these treatments obtained a range of removal of TPH from 10 to 20%, which were the lowest of all the treatments. On the other hand, in the BAECH1500 bioreactor the highest temperatures were originated, reaching in the third week 34 ºC which coincides with the highest removal of TPH in the treatments, which confirms that the highest temperatures obtained in the treatments are originated for the highest percentages of removal as a result of increased microbial activity.

Conclusions

The incorporation of HA into biological reactors significantly increases the TPH removal from the OBDC sample containing an initial concentration of 116,372 mg/kg. The addition of 1,500 mg/L solution of HA (BAECH1500) allowed to get the highest TPH removal (72%), also. In this bioreactor, it was registered the higher temperature and the largest production of CO_2, these facts suggest that the HA enhance the solubilization of the pollutants and increase the activity of the biological system in consequence. The concentration of HA in BAECH1500 is below the CMC concentration, indicating that lower concentration than CMC can be successfully used for biological treatment of OBDC samples.

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