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Bioavailability and dissipation of anthracene from soil with different alkalinity and salinity

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Abstract

Bioavailability of contaminants, such as anthracene (Anthra), a polycyclic aromatic hydrocarbon (PAHs), and their removal from soil has been related to their extractability with non-exhaustive techniques, such as hydroxypropyl-beta-cyclodextrin (HPCD) or n-butanol. Anthra was extracted with HPCD, n-butanol and by exhaustive ultrasonic extraction method from sterilized and unsterilized alkaline soil of the former lake Texcoco, having pH ranging from pH 8.2 to 10.1 and electrolytic conductivity varying from 1.2 dS m^{-1} to 95.2 dS m^{-1}, respectively. About 24.4 and 37.6% of Anthra was removed biologically from soil as estimated by exhaustive technique after 56 days. The percentage of Anthra that was removed from soil by exhaustive technique was not related to the amount that was extractable with HPCD or n-butanol. It was found that the Anthra extractable with n-butanol or HPCD did not correlate well with the removal of the contaminant from soil. In this study, the removal of Anthra from soil could not be predicted by the amount of Anthra that was extracted with n-butanol or HPCD

Key words

Hydroxypropyl-beta-cyclodextrin, n-Butanol; Polycyclic aromatic hydrocarbon, Soil characteristics

Introduction

Contamination of soil with polycyclic aromatic hydrocarbons (PAHs) occurs often and their removal is difficult and time consuming. Polycyclic aromatic hydrocarbons are produced naturally, such as in forest fires and volcanic eruptions, or as a result of human activity such as fossil fuel burning, production of gas and coal tar and wood processing. It is well known that soil microorganisms can degrade PAHs. Both bacteria and fungi have been described to degrade PAHs, but the mineralization rate is often low. In general, the rate of degradation of PAHs decreases with increased number of aromatic rings, but is also affected by soil characteristics and that in two different ways (Chung and Alexander, 1998; Bogan and Sullivan, 2003). First, soil properties can inhibit microbial activity and as such degradation of PAHs. For instance, high salinity is known to inhibit carbon and nitrogen mineralization and soil enzyme activities, which are crucial for decomposition of organic matter. This reduction in activity might reduce removal of PAHs from soil. Second, soil characteristics determine the bioavailability of contaminants in soil. It is well known that contaminants can be physically immobilized as they diffuse into micropores of soil particles and can be fixed or react with soil organic matter, rendering them unavailable for microbial degradation (Nam and Alexander, 1998; Macleod and Semple, 2000; Yang *et al*., 2011).

Soil of the former lake Texcoco (Mexico) is extreme by alkaline saline with pH that can reach 10.5 and electrolytic conductivity (EC) often above 100 dS m^{-1} (Dendooven *et al.,* 2010). Removal of PAHs from these soils occurred, but was highly variable and not related to the alkalinity or salinity of the soil or microbial activity as evidenced by emission of carbon dioxide. It appeared that physical or chemical soil characteristics other than alkalinity or salinity, such as organic material, might have determined the bioavailability and removal of PAHs from soil (Chung and Alexander, 2002; Bogan and Sullivan, 2003).

Different techniques have been used to define the bioavailability of PAHs in soil, such as extraction with organic solvents, which will control their removal. Two non-exhaustive

extraction techniques, hydroxypropyl-beta-cyclodextrin (HPCD) (Reid *et al.,* 2000) and *n*-butanol were used as solvent (Kelsey *et al.*, 1997) to determine the bioavailability of an organic contaminant, *i.e.* anthracene (Anthra), in four soils of Texcoco with varying pH and EC. *n*-Butanol was selected as the amount of PAHs extracted with it that has been found to be related to the availability of PAHs for earthworms and bacteria (Kelsey *et al.,* 1997), while HPCD has been shown to predict the microbial degradation of a range of compounds in a range of soils (Swindell and Reid, 2006). An exhaustive ultrasonic extraction method, based on the technique of Song *et al*. (1995), was used to measure the removal of Anthra from these soils and related to the bioavailability of the organic contaminant in soil, as determined with *n*-butanol or HPCD. The objective of this study was to investigate the relationship between the removal of Anthra from soils with different salinity and alkalinity, as determined with an exhaustive technique and its extractability with two non-exhaustive techniques.

Materials and Methods

Site description and soil sampling : The sampling sites were located in the former lake Texcoco in the valley of Mexico City. Five 0.5-ha plots were defined at four sites with different electrolytic conductivity (EC). Soil with EC 1.2 dS m^{-1} was considered as soil A, with EC 3.2 dS m^{-1} as soil B, with EC 80.2 dS m^{-1} as soil C and with 95.2 dS m^{-1} as soil D. Soil was sampled at random by augering 0-15 cm top-layer of the five plots at each of the four sites. The soil from each plot was pooled so that five soil samples were obtained (n = 5) for each site. As such, 20 soil samples were obtained. This field based replication was maintained during the incubation study.

Soil preparation : The soil was taken to the laboratory and treated as follows. The soil from each site was passed separately through a 5 mm sieve and adjusted to 40% water holding capacity (WHC) by adding distilled water. The soil was conditioned at ambient room temperature (22±2°C) in drums containing a beaker with 1 M NaOH to trap CO_2 evolved and a beaker with 1 l water to avoid desiccation for a week.

Fourteen sub-samples of 20 g of soil from each plot (n = 5) and site with different EC (n = 4) were added to 120 ml glass flasks. Half of them (seven) were sterilized on three successive days. The sterilized and unsterilized soil samples were amended with Anthra dissolved in 2 ml acetone under sterile conditions. As such, approximately 550 mg Anthra kg^{-1} was added to soil. This concentration allows to study the removal of Anthra from soil and the factors controlling it and survival of earthworms was not affected (Contreras-Ramos *et al.,* 2006). After spiking the soil with Anthra, all samples were placed under vacuum in a desiccator for 45 min to evaporate acetone.

The flasks were placed in 945 ml glass jars containing 10 ml distilled water to avoid desiccation of the soil and a vessel with 20 ml 1 M NaOH to trap the evolved CO_2. The jars were sealed and stored in dark at 22±2°C. In addition, 18 jars containing a vessel with 20 ml 1 M NaOH and 10 ml distilled water, but without soil, were sealed and served as control to account for the CO_2 trapped from air. After 1, 3, 7, 14, 28 and 56 days, a flask of each plot was chosen at random from each treatment and opened. The vessel with NaOH was removed and 1.5 g soil was weighted and extracted for Anthra with an exhaustive extraction procedure (Song *et al.,* 1995), 1.5 g was weighted and extracted for Anthra with HPCD and 10 g was weighted and extracted for Anthra with *n*-butanol.

Soil chemical analysis and extraction of anthracene : Soil pH, total N, organic C and distribution of soil particle size were determined as described by Contreras-Ramos *et al*. (2006). Concentration of Anthra in soil was determined by exhaustive ultrasonic extraction method developed by Song *et al*. (1995) and described by Contreras-Ramos *et al*. (2006).

Concentration of Anthra in soil was determined using a non-exhaustive extraction with HPCD (Reid *et al.,* 2000) and *n*-butanol (Kelsey *et al.,* 1997). Briefly, 1.5 g sub-sample of soil was amended with 25 ml 60 mM HPCD, shaken in dark for 20 hr and centrifuged at 3000 rpm for 10 min. The supernatant was mixed with 20 ml dichloromethane and shaken for 10 min. The HPCD was decanted and the solution centrifuged at 3000 rpm for 10 min. The remaining HPCD was discarded and dichloromethane was evaporated in dark overnight at room temperature. Two ml of acetone was added to the residue, mixed and analyzed for Anthra. A solution of HPCD with known concentration of Anthra was used to determine the amount of Anthra that was lost during the procedure at each sampling day. The amount of Anthra lost during the procedure was approximately 20%, so data reported were adjusted for these losses. A 10 g sub-sample of soil was extracted with 15 ml *n*-butanol and shaken for 5 min. The soil *n*-butanol mixture was left to stand for 30 min and centrifuged at 3000 rpm for 10 min. The supernatant was decanted and evaporated in dark at room temperature. Two ml acetone was added to the residue and analyzed for Anthra. A solution of *n*-butanol with known concentration of Anthra was used to determine the amount of Anthra that was lost during the procedure at each sampling day. Although, the amount of Anthra lost during the procedure was <2%, data reported were adjusted for these losses.

Statistical analysis : Cumulative production of CO_2 was regressed at elapsed time using a linear regression model which was forced to pass through the origin, but allowed different slopes (production rate) for each treatment. Concentrations of Anthra, as determined with different extraction techniques in sterilized and unsterilized soil were subjected to one-way analysis of variance using PROC GLM (SAS Institute, 1989) to test for significant differences between the soils and then least significant difference was calculated.

Results and Discussion

About 92.4 to 100.5 of 550 mg Anthra kg^{-1} added to sterile soil was recovered by exhaustive extraction technique just after contaminating the soil and was found to be significantly different between the soils (Table 1). The amount of Anthra extracted from sterile soil by exhaustive technique did not change significantly over time (Fig. 1a). After 56 days, 90.2 to 105.5% of the added Anhtra was extracted from sterilized soil and was found to be significantly different between soils (Table 1).

The concentration of Anthra extracted from unsterilized soil by the exhaustive technique decreased sharply in soil C within first day and showed little difference thereafter (Fig. 1b). In soil D, concentration of Anthra decreased til day 7, but showed little difference thereafter. In soil A and B, the concentration of Anthra decreased only after day 28. After 56 days, 62.4 to 75.6 % of the added Anthra was removed from unsterilized soils (Table 1).

The concentration of Anthra extracted from sterilized soil with *n*-butanol were similar for different soils and showed the same pattern (Fig. 1c). The amount of Anthra extractable with *n*-butanol nearly halved within the first 14 days and showed little difference afterwards in soil A and C, but further decreased in soil B and D. The amount of Anthra extracted with *n*-butanol from sterilized soil after 56 days ranged from 32.6 % in soil A to 57.2% in soil C (Table 1), respectively.

The concentration of Anthra extracted from unsterilized soil with *n*-butanol was more than half between the onset of the experiment and day 7 (Fig. 1d). Subsequent decreases were small with least found in soil A and highest in soil B and D. At day 56, the amount of Anthra extracted with *n*-butanol from unsterilized soil ranged from 18.4 % in soil B to 50.3 % in soil A and was significantly different between the soils ($P < 0.05$) (Table 1).

The concentrations of Anthra extracted with HPCD were significantly different between sterilized soils just after application and ranged from 20.4 to 73.6 % ($P < 0.05$) (Table 1, Fig. 1e). After 56 days, the amount of Anthra extracted with HPCD from sterilized soils were significantly different and ranged from 11.1 % in soil C to 33.3 % in soil A ($P < 0.05$), respectively.

The concentration of Anthra extracted from unsterilized soil with HPCD were <160 mg Anthra kg^{-1} and significantly different between the soils ($P < 0.05$) (Fig. 1f). After 56 days, the lowest amount of Anthra was extracted from soil C (2.0 %) and the highest from soil A (27.5). The amount of Anthra extracted with HPCD was less than that extracted with *n*-butanol or by exhaustive technique.

None of the soil characteristics were correlated significantly with the percentage of Anthra extracted by exhaustive technique from sterile soil on day 0 and 56 and from unsterilized soil on day 56 (No data shown). However, the percentage of Anthra extracted with *n*-butanol from unsterilized soil after 56 days was negatively and significantly correlated with pH, WHC, clay content and CO_2 emission, and positively with sand content ($P < 0.05$). The percentage of Anthra extracted with HPCD from unsterilized soil after 56 days was negatively and significantly correlated with pH, WHC and CO_2 emission ($P < 0.05$).

The exhaustive method as suggested by Song *et al*. (1995) is a robust technique as most of the Anthra added was extracted immediately after application (Table 1). Song *et al*. (2002) reported 93% recovery of Anthra from soil with 98% sand.

The amount of Anthra extracted from sterilized soil by the method of Song *et al*. (1995) showed only small changes over time. This indicated that only small amount of Anthra was sequestered in soil and the effect of abiotic processes on the concentration of contaminant was negligible. Kottler and Alexander (2001) reported similar results and found that 101.7 % of Anthracene was recovered by exhaustive extraction technique (Soxhlet) from sterilized soil shortly after contamination and 97.8 % after 28 days. Extractability and bioavailability of PAHs in soil decreases as the soil-PAH contact increases over time. This phenomenon has been termed as 'ageing effect' (Hatzinger and Alexander, 1995) and is determined mostly by soil and PAHs characteristics, and the length of conditioning (Chung and Alexander, 2002). In view of short incubation time in the present study, the incubation time was short so the amount of Anthra sequestered in Texcoco soil was also low.

Since the amount of Anthra sequestered in different soils was low so most of the Anthra was biologically removed from

Table 1 : Percentage anthracene extracted from sterile soil as a percentage of 550 mg added at day 0 and 56, and unsterilized soil after 56 days

Soil	Extracted from sterilized soil day 0	Extracted from sterilized soil at day 56	Extracted from soil at day 56
Extracted with an exhaustive technique			
Soil A	94.8 ±5.7	105.5 ±16.6	72.1 ±12.6
Soil B	97.4 ±18.6	90.2 ±15.0	75.6 ±14.1
Soil C	92.4 ±20.1	101.3 ±18.3	62.4 ±8.3
Soil D	100.5 ±6.1	94.1 ± 9.7	71.1 ±6.8
Extracted with n-butanol			
Soil A	101.0 ±9.4	55.2 ±18.0	50.3 ±13.2
Soil B	103.5 ±20.4	43.2 ±16.2	18.4 ±5.5
Soil C	92.3 ±19.6	57.2 ±13.4	25.7 ±11.9
Soil D	97.0 ±30.4	32.6 ±14.9	18.5 ±5.4
hydroxypropyl-beta-cyclodextrin			
Soil A	34.1 ±11.6	33.3 ±9.5	27.5 ±3.3
Soil B	73.6 ±12.2	11.1 ±4.5	11.1 ±2.5
Soil C	20.4 ±3.6	24.6 ±4.4	2.0 ±4.0
Soil D	22.9 ±7.7	18.3 ±12.2	13.2 ±7.8

Values are mean of five different soil samples ±SD

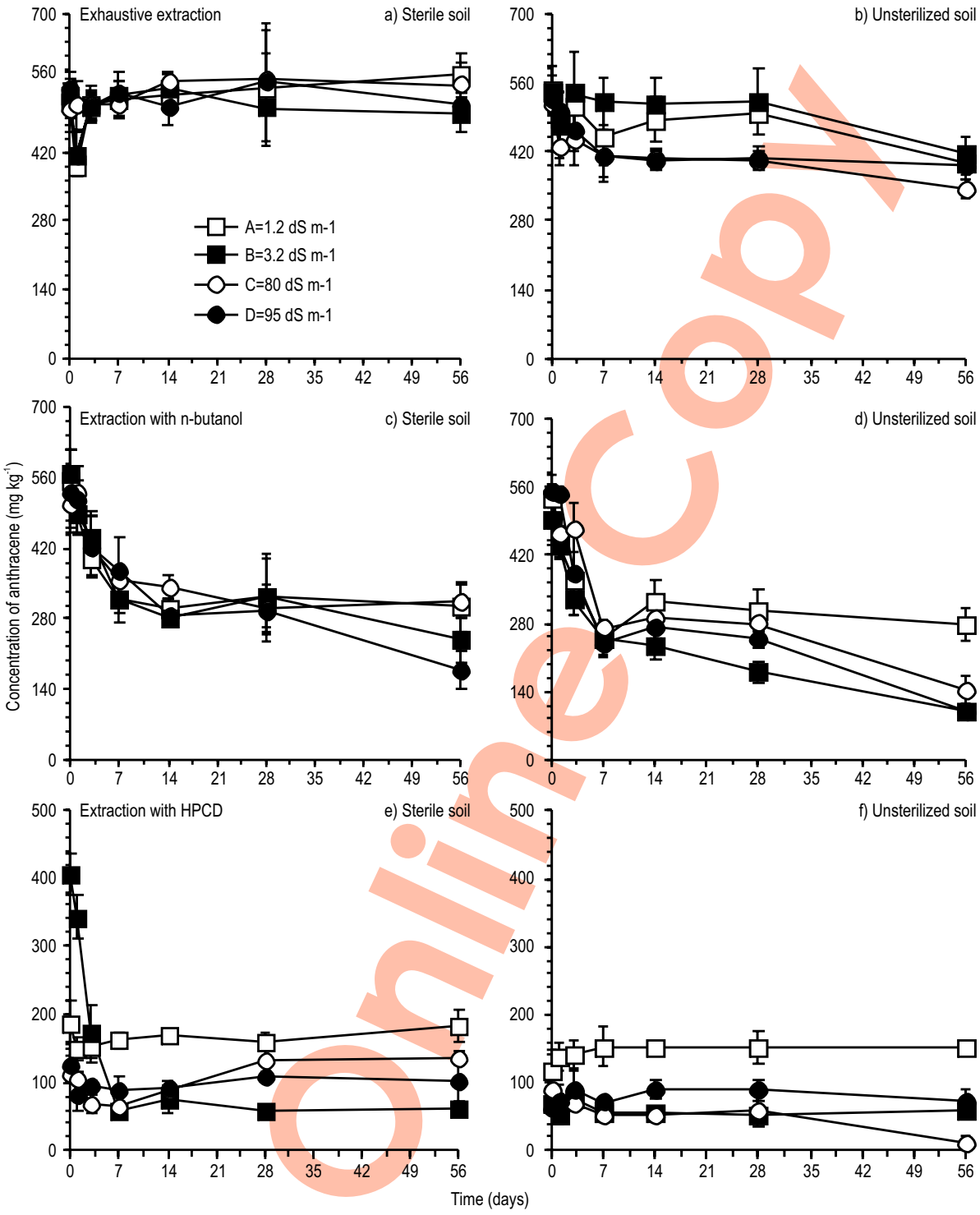

Fig. 1 : Amount of anthracene (mg kg^{-1} dry soil) extracted by exhaustive technique (Song *et al.,* 1995) from a) sterilized soil and b) unsterilized soil with electrolytic conductivity 1.2 dS m^{-1} (□), 3.2 dS m^{-1} (■), 80 dS m^{-1} (○) or 95 dS m^{-1} (●), with n-butanol from c) sterilized soil and d) unsterilized soil, and with hydroxypropyl-beta-cyclodextrin (HPCD) from e) sterilized or f) unsterilized soil. Soil was incubated at 22 ± 2°C for 56 days. Bars represent ± SD values; values were mean of five replicate

them. It is well known that soil microorganisms can remove hydrocarbons from soil and numerous bacteria and fungi have been reported to degrade PAHs (Amezcua-Allieri *et al.,* 2012). Biological removal of PAHs depends on its composition, more aromatic rings, more resistant the compound is. Soil characteristics are also known to affect removal of Anthra from soil. It was hypothesized that high pH and large salt content would inhibit biotic removal of Anthra from Texcoco soil. However, no such effect was found in this experiment. The amount of Anthra biologically removed was similar in soil A and D, although the pH and EC were much higher in soil D than in soil A.

Although extraction of Anthra from soil with *n*-butanol is considered less exhaustive than the method suggested by Song *et al*. (1995), nearly all the Anthra added to four Texcoco soils was extractable immediately after application. Similar results were reported by Swindell and Reid (2006). After 1 day, nearly all of the added phenanthrene was extracted from contaminated soil with *n*-butanol. However, Kottler and Alexander (2001) could recover only 64.7 % of the Anthra added to sterilized soil.

The dynamics of Anthra extracted with *n*-butanol from sterilized soil was different from the Anthra extracted by exhaustive method. The amount of Anthra extracted with *n*-butanol from sterile soil showed a sharp drop within first few days and after 56 days, while approximately half of the Anthra added was not extractable from sterile soil with *n*-butanol although nearly 100% was extractable by exhaustive technique. A mild extraction with *n*-butanol has been suggested as an appropriate way to determine the bioavailability of a hydrocarbon in soil (Kelsey *et al.,* 1997). A sharp drop in the amount of Anthra extractable with *n*-butanol within first two weeks, as found in the present study, might be an indication of a drop in its bio-availability.

The amount of Anthra extracted from sterilized soil with HPCD was low (Table 1). Swindell and Reid (2006) reported near by 100% recovery of phenanthrene one day after application in two soils with <10% clay, but only 53.8 % recovery from soil with 20% clay, and suggested that organic matter and soil type influenced the amount of phenanthrene extracted using HPCD. Reid *et al*. (2000) found that in sandy soil, the extractability was 89.1 % while only 74.8 % in clayey soil rich in organic matter. However, in the present study, no relationship with clay or organic matter content and extractability of Anthra with HPCD was found.

The ability of *n*-butanol to predict the removal of Anthra from soil as determined by exhaustive technique was poor. These results confirmed the results obtained by Chung and Alexander (1998), Tang and Alexander (1999) and Bogan and Sullivan (2003). The latter stated that 'although *n*-butanol extraction under rapid, mild conditions may represent an excellent means for estimating PAH availability to earthworms, and possible other receptor macro-organisms (Tang and Alexander, 1999), it was considerably less useful for estimating bacterial biodegradability'. The ability of HPCD extractability of Anthra to predict its removal from soil by exhaustive technique was also poor. As such, although HPCD extraction of Anthra might represent an excellent means for estimating microbial degradable fraction, it was less successful for estimating the amount of Anthra removed from soil.

None of the measured soil characteristics was correlated to biological removal of Anthra from soil as determined by exhaustive technique. However, soil organic matter has often been found to affect dissipation of PAHs from soil (Yang *et al.,* 2010), but not in the present study. It can be speculated that soil characteristics other than those measured, e.g. type of clay minerals, defined the removal of Anthra from soil. Consequently, it might be difficult to determine which factor affected the dissipation of organic contaminant from soil.

The percentage of Anthra extracted with *n*-butanol and HPCD from unsterilized soil after 56 days, however, were highly significantly and negatively affected by pH and WHC ($P < 0.001$). As such, extractability of Anthra with *n*-butanol or HPCD reduced with increased WHC of the soils (Cachada *et al*., 2012). The WHC of a soil is normally related to the clay content, aggregate formation and soil organic matter content and those factors are known to affect the extractability of organic contaminants from soil. An increase in pH reduces microbial activity and this might have inhibited the removal of Anthra from soil as defined with HPCD and *n*-butanol.

It can be debated whether the Anthra that was not extractable with *n*-butanol or HPCD poses a threat to the environment. The amount of Anthra extractable with *n*-butanol or HPCD might be a better indicator of the Anthra that might pose a threat to the environment than the amount extracted with an exhaustive technique. However, it was also clear from this study that some of the Anthra that was not extractable with *n*-butanol or HPCD became available for degradation and was removed from soil.

It was found that the removal of Anthra was due to biological degradation, as abiotic sequestration was low or non-existent. There was no correlation between the Anthra biologically removed from soil and the amount that was available as defined with *n*-butanol or HPCD.

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