



STUDY OF TOLUENE DEGRADATION KINETICS IN A FLAT PLATE VAPOR PHASE BIOREACTOR USING OXYGEN MICROSENSORS

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ABSTRACT

The paper describes the toluene degradation process in a flat plate vapor phase bioreactor (VPBR) by a *Pseudomonas putida* 54G biofilm. Oxygen microelectrodes were used to measure oxygen concentration profiles through the gas, liquid, and biofilm phases. The linear shape of the dissolved oxygen concentration profile in the outer 87% of the biofilm thickness suggested an absence of reaction in this layer. Oxygen consumption in the remaining basal 13 % (0.3 mm) followed zero order kinetics with a rate constant of $102.2 \text{ g m}^{-3} \text{ h}^{-1}$, for toluene gas concentration of 1.5 g m^{-3} .

The increase in respiratory activity near the substratum was confirmed by microscopic study of cryogenic biofilm sections, and the lack of activity in the surface film was interpreted as a consequence of injury exerted by the toxic substrate. The accumulation of dead cells on the top of the biofilm contributed a resistance to the transport of substrates to deeper layers of the biofilm suggesting a protective role of the outer layer against the harmful effect of the toxic. These results highlight a new conceptual biofilm model in which both microbial growth and inactivation are controlled by substrate transport, leading to a structure that itself controls substrate availability. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Biofilms, microelectrodes, bioremediation, Volatile Organic Compound (VOC), Vapor Phase Bioreactor (VPBR).

INTRODUCTION

The application of biofilters and vapor phase bioreactors (VPBRs), for degrading volatile organic compounds (VOCs) is becoming a common practice due to low cost and good performance. To identify the controlling step of the overall degradative process, it is critical to determine the relative importance of mass transfer, kinetics and stoichiometry. Diks and Ottengraf (1991) found that the gas-liquid mass transfer resistance was negligible in a trickling filter degrading dichloromethane. They also reported that the degradation process inside the biofilm was the rate limiting step. Nevertheless, Kirchner *et al.* (1987) reported that the mass transfer through the liquid film around activated carbon grains was the rate limiting step during the degradation of solvents (aldehydes, methyl ethyl ketone and ethyl acetate) in a trickle bed reactor. The same conclusion was reached later using different pollutants as acetone, propionaldehyde, naphthalen and toluene (Kirchner *et al.* 1989). Different observations were made by Partidario and Carrondo (1993) who reported that the overall conversion rate of trimethylamine in a packed bed bubble bioreactor was controlled by diffusion from the gas to liquid phase. Sotoudeh and Windsperger (1994) concluded that the biological gas purification process in a trickling filter bed reactor was limited by the mass transfer through the liquid layer surrounding the packing material for low gas concentrations, and by the biological reaction within the biofilm for high gas concentration.

All these observations were assumed to occur under non-oxygen-limited conditions. However, the stoichiometric requirement for oxygen in the catabolic degradative reaction of VOCs usually range between three and four grams of oxygen per gram of VOC, which means that in many practical situations the overall process is controlled by the oxygen availability (Leahy and Colwell, 1990; Sturman *et al.* 1995). In addition, the transport of oxygen through the different phases involved in the process may also limit the degradative process, specially when three phases -vapor, liquid, and biofilm- are involved.

Oxygen microsensors have been used to determine respiration rates in biofilms (Bungay 1969; Lewandowski *et al.* 1991), as well as for describing the mass transfer through gas-liquid interfaces (Lee *et al.* 1978). Therefore, oxygen microsensors can be used to determine the limiting step of the overall degradation process when oxygen is the limiting substrate. The goal of this work is to describe the mechanisms of toluene degradation by a *Pseudomonas putida* 54G biofilm in a flat plate vapor phase bioreactor (FPVPBR). Mass transport rates through the gas, liquid and biofilm phases and interfaces were evaluated, as well as the oxygen utilization rate within the biofilm.

MATERIALS AND METHODS

Flat Plate Vapor Phase Bioreactor (FPVPBR).

The flat plate reactor (Figure 1) has been described in detail elsewhere (Mirpuri 1996). The gas feed stream was prepared by mixing humidified air with toluene-saturated air, and delivered into the FPVPBR at a flow rate of 50 ml/min. Sterile HCMM2 mineral salts medium (Ridgway *et al.*, 1990) flowed through the reactor at a constant flow rate of 1 ml/min. Both liquid and vapor flow regimes were laminar flow, and the reactor was operated in countercurrent mode. In Table 1 the operation conditions for the FPVPBR are presented. The sole external carbon and energy source for bacterial degradation was toluene introduced in the vapor phase.

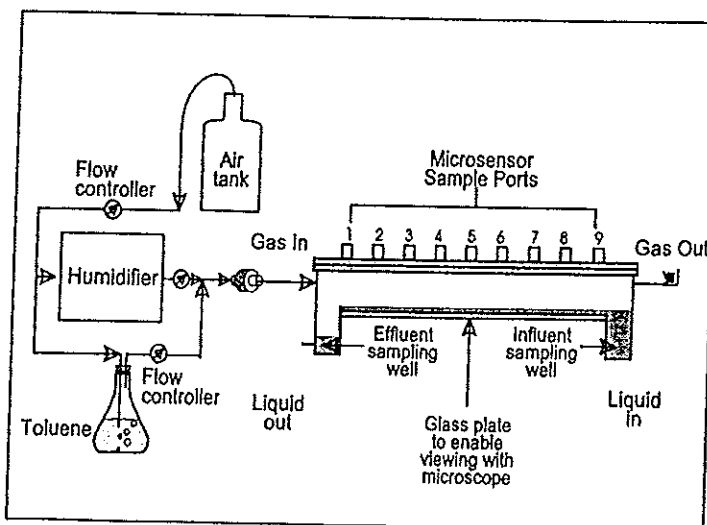


Figure 1. Scheme of the Flat Plate Vapor Phase Bioreactor (FPVPBR).

Two sets of experiments were conducted.

In the first, gas phase influent toluene concentration was set at 150 ppm (0.47 g m^{-3}). Under these conditions, toluene was the limiting substrate and oxygen was present in excess. In the second experiment, gas influent toluene concentration was 750 ppm (2.37 g m^{-3}), making oxygen the limiting substrate within the biofilm. Experiments were run at constant room temperature of 25°C . During start-up, suspended cells of *Pseudomonas putida* 54G, a toluene-degrading bacterium isolated from a gasoline contaminated aquifer at Seal Beach, California, were introduced into the reactor and recycled for 12 hours, after which the reactor was operated continuously in a once-through mode. Biofilm growth was observed daily until steady state was reached, as indicated by relatively constant substrate removal rate, biofilm thickness, and density, about one month after start-up. Once the steady state was reached, several dissolved oxygen profiles were taken through the different ports along the reactor. Biofilm samples were also taken for measuring total and respiring cell densities by staining techniques.

Table 1. Operation conditions for the flat plate vapor phase bioreactor.

Phase	Gas	Liquid
Flow rate ($10^{-3} \text{ m}^3 / \text{h}$)	3.0	0.06
Fluid superficial velocity (m / h)	1.87	0.37
Residence time in the glass plate (h)	0.21	1.07

Oxygen microsensors

Oxygen probes were fabricated as described previously (Revsbech, 1989, and Revsbech and Jorgensen 1986). Tips of the oxygen probes were about $10 \mu\text{m}$ in diameter, which allowed for non-invasive measurements of oxygen concentration in the biofilm. Probes were calibrated in air-saturated water and nitrogen-saturated water at 25°C . Effect of stirring on the microsensors was less than 5% of the response and the response time less than one second.

Probes were mounted on computer controlled micro-manipulators, and used for *in-situ* measurement of oxygen in the liquid and biofilm phases through the sampling ports. An inverted microscope (Olympus) was used to locate the tip of the probe, and to determine the position of the biofilm-liquid interface. Oxygen concentration profiles obtained within the biofilm were fitted to the traditional diffusion-reaction model using a nonlinear optimization method. The consumption rate of oxygen within the biofilm was assumed to follow a Monod type expression. Mathlab (Math Works Inc.) computer software was used to carry out the optimization procedure.

Biofilm analysis

Biofilm samples taken at several points along the reactor were analyzed for total and respiring cells by direct epifluorescent microscopy. Samples were counterstained by DNA-binding fluorochrome 2,4-diamidino-2-phenylindole (DAPI) and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) based on a procedure outlined by Rodriguez *et al.* (1992) with minor modifications. We refer to DAPI and CTC stained cells as total and respiring cells respectively. Microscopic examination was done using an Olympus BH2-RFCA epifluorescent light microscope (Scientific Instrument Company, UT) with a UV100X (refractive index=1.3) oil immersion lens.

Toluene Sampling

Gas and liquid samples were taken from the influent and effluent, and through the sampling ports at several points along the reactor length, and analyzed for toluene concentration using gas chromatography; (GC)

model HP 5890 Series II GC equipped with a FID and an Alltech 0.1% A1-1000 Graphpac GC 80/100, 6' x 1/8" x 0.085", S.S. column.

RESULTS AND DISCUSSION

Oxygen requirements

Figure 2 shows five oxygen concentration profiles through the liquid and biofilm phases measured for different toluene gas concentrations. It can be seen that the oxygen became limiting for toluene gas concentration of 1.50 g m^{-3} , which corresponds to a toluene liquid concentration of 7.89 g m^{-3} (for a value of Henry's constant for toluene of 0.19, determined experimentally). The biofilm thickness ranged from 1.4 to 2.3 mm. The linear shape of the oxygen concentration profiles suggested that the transport of substrates was governed by molecular diffusion. Consequently Fick's law was used to calculate the flux of substrate into the biofilm.

It was expected that the toluene was completely depleted for toluene gas concentrations lower than 1.5 g m^{-3} , consequently, under non-limiting conditions for oxygen we assumed zero concentration for toluene at the substratum. A linear pattern for the toluene concentration profile in the liquid and biofilm phases, and a diffusion coefficient for toluene in the biofilm 58.2 % of the value in water ($2.87 \times 10^{-6} \text{ m}^2 \text{ h}^{-1}$) were assumed as in the case of oxygen (see last section of this paper). The toluene flux into the biofilm surface, J_b , was estimated from Equation 1, where C_{sat} is the saturation concentration at the gas-liquid interface, D_L and D_b are the diffusion coefficients for toluene in water and biofilm respectively, and L_L and L_b are the thickness of the liquid layer and biofilm respectively.

$$J_b = C_{sat} \cdot \frac{D_L D_b}{D_b L_L + D_L L_b} \quad (1)$$

In Figure 3 the fluxes of oxygen (experimental) and toluene (Equation 1) and their ratio are plotted for different toluene gas concentrations. Since transport of oxygen and toluene in the axial direction was at least two orders of magnitude smaller than the transport by molecular diffusion in the normal direction within the biofilm, it can be assumed that the flux ratio represents the total amount of oxygen consumed per mass unit of toluene degraded, i.e. the stoichiometry of the overall process. This flux ratio was close to the stoichiometry of the anabolic reaction, $0.69 \text{ g O}_2 / \text{g toluene}$ (Figure 3) for toluene concentrations of 0.23 g m^{-3} , and rapidly increased to $3.2 \text{ g O}_2 / \text{g toluene}$ for a toluene gas concentration of

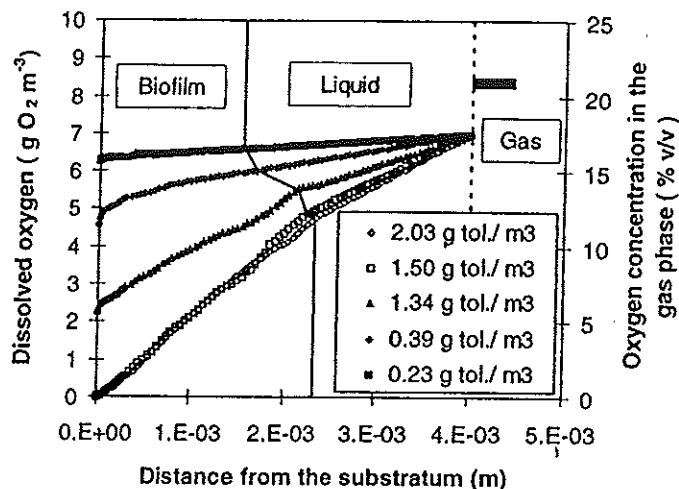


Figure 2. Oxygen concentration profiles through gas, liquid, and biofilm phases for different toluene gas concentrations.

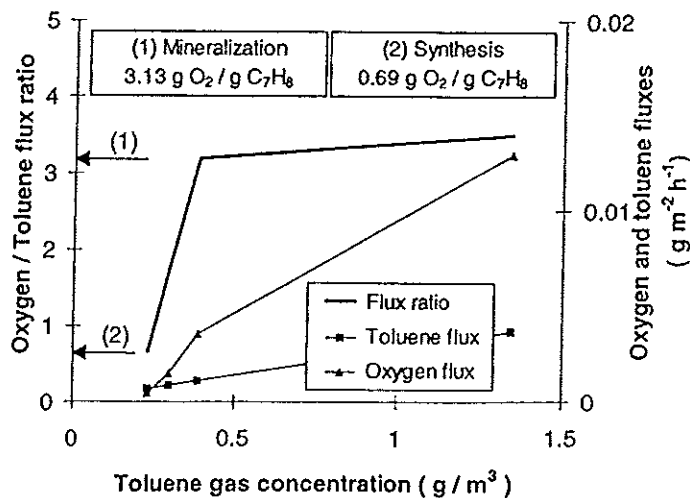


Figure 3. Local fluxes of oxygen (experimental) and toluene (estimated), and their ratio for different toluene gas concentrations. Toluene was the limiting substrate.

0.39 g m^{-3} . The maximum ratio was 3.5 g/g , which is slightly over the value predicted by the stoichiometry of the mineralization reaction, ($3.13 \text{ g O}_2 / \text{g Toluene}$) suggesting that part of the oxygen may be consumed by processes not associated with toluene degradation. These observations must be considered only qualitatively since the toluene fluxes can be either underestimated or overestimated. Nevertheless, there appears to be a tendency of the process towards cell growth at low concentrations and towards mineralization at high concentrations.

Respiratory activity stratification

The oxygen concentration profiles Figure 2 exhibited an increasing oxygen concentration gradient near the substratum. This phenomenon is more clearly seen in the totally penetrated biofilm situations when toluene is the limiting substrate. In Figure 4, the oxygen concentration profile within the biofilm was presented for a toluene gas concentration of 0.39 g m^{-3} . The oxygen concentration gradient was $7.0 \times 10^2 \text{ g m}^{-4}$ above 0.3 mm from the substratum, and increased by 17 times to a value of $1.19 \times 10^4 \text{ g m}^{-4}$ below 0.03 mm from the substratum. Since visual observation of the biofilm structure under the inverted microscope revealed that the biofilm was very homogeneous, it is unlikely that large changes in the diffusion coefficient were responsible for the increased gradient. Consequently, such an increase in the oxygen concentration gradient suggests a gradient in the respiratory activity within the biofilm.

The high sensitivity of the biofilm to mechanical stress made possible the analysis of the upper layer of the biofilm when tilting the reactor to remove the upper layer of the biofilm. The cell numbers for respiring and total cells within the biofilm before and after the sloughing event revealed that the upper layer was formed mostly of dead cells (90 % dead cells), and the base biofilm was very active with 70 % respiring cells. These observations were confirmed by epifluorescence microscopy of cryogenic biofilm sections (Huang *et al.* 1995) previously counterstained by DAPI and CTC, which revealed a two-layer biofilm, with a thin ($0.03\text{-}0.1 \text{ mm}$) layer of respiring cells overlaid by a layer of mostly non-respiring cells. A possible explanation for such a phenomenon is supported by recent studies of the physiological changes of *P.*

putida cells degrading toluene during prolonged exposure to different concentrations of the toxic substrate (Mirpuri, 1996). The number of non-respiring cells and mutants increased with increasing the toluene concentration and time, to reaching values two orders of magnitude higher than the number of cells degrading the toluene. This observation is in accordance with the accumulation of inactive cells on the top of the biofilm where the toluene concentration is higher.

Mass transport and reaction

To study the mass transport and reaction processes taking place within the biofilm under oxygen limiting conditions, we used a representative oxygen concentration profile through the gas, liquid and biofilm phases for a toluene gas concentration of 1.50 g m^{-3} (Figure 5). Similar results were obtained for higher toluene concentrations. The biofilm thickness was 2.3 mm and the thickness of the liquid layer above the biofilm

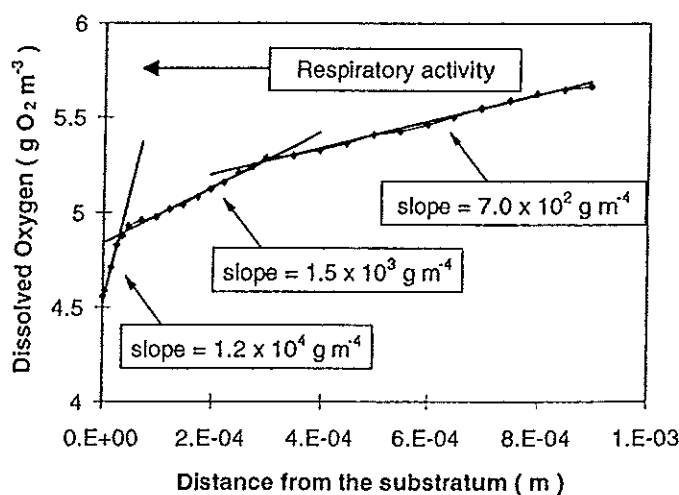


Figure 4. Oxygen concentration profile within the biofilm for a toluene gas concentration of 0.39 g m^{-3} . The different slopes represent the concentration gradient change with depth.

was 1.7 mm. The linear shape of the concentration profile in the liquid and outer biofilm suggested that the transport of substrates was governed by molecular diffusion. Therefore, the flux of oxygen through the biofilm surface was evaluated from Fick's law applied at the liquid side of the biofilm-liquid interface (Figure 5) and using the diffusion coefficient of oxygen in water, $9.0 \times 10^{-6} \text{ m}^2 \text{ h}^{-1}$. The resulting flux was $0.010 \text{ g m}^{-2} \text{ h}^{-1}$. This value must be equal to the oxygen flux at the biofilm side of the biofilm-liquid interface to satisfy flux continuity. The effective diffusion coefficient for oxygen within the biofilm was calculated from the ratio of the oxygen gradients. The value obtained was $5.22 \times 10^{-6} \text{ m}^2 \text{ h}^{-1}$, which is the 58.2 % of the molecular diffusivity for oxygen in water ($9.0 \times 10^{-6} \text{ m}^2 \text{ h}^{-1}$).

The oxygen concentration profile within the basal 0.3 mm of the biofilm was fitted to the traditional diffusion-reaction model assuming homogeneous biofilm and Monod kinetics for oxygen consumption within the biofilm. The results revealed zero order kinetics for the oxygen uptake within this portion of the biofilm (K_s values of zero). The value obtained for the kinetic constant was $102.2 \text{ g m}^{-3} \text{ h}^{-1}$. According to Harremoës (1978) the flux of oxygen into the biofilm is equal to the surface uptake rate, which for a fully penetrated biofilm can be calculated by multiplying the zero order kinetic constant by the biofilm thickness. Therefore, the active biofilm thickness can be calculated by dividing the Fickian value of the oxygen flux by the zero order kinetic constant. The value obtained was 0.1 mm, which compared to a total thickness of 2.3 mm would result in a biofilm only 4% active, which for zero order kinetics would correspond to the effectiveness factor in the biofilm (Andrews, 1988). However the concentration profile (Figure 5) shows a 120 % increase on the oxygen gradient at 0.3 mm from the substratum, taken as the active thickness of the basal biofilm.

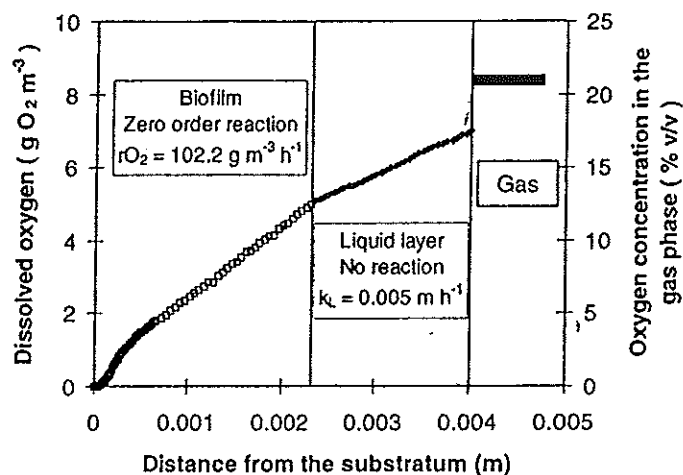


Figure 5. Oxygen concentration profile through gas, liquid, and biofilm phases for a toluene gas concentration of 1.5 g m^{-3} .

$$\Phi^2 = \frac{k^0 L_a}{2C_s \left(\frac{D_b}{L_b} \right)} \quad (2)$$

To evaluate the implications of this stratification of activity on the overall process we calculated the generalized Thiele modulus for Monod kinetics, Φ . For zero order kinetics the modulus can be calculated following Equation 2, where k^0 is the zero order kinetic constant ($102.2 \text{ g m}^{-3} \text{ h}^{-1}$), L_b is the total biofilm thickness (0.0023 m), C_s is the substrate concentration at the biofilm surface (5.02 g m^{-3}), L_a is the active biofilm thickness (m), and D_b is the diffusion coefficient of the substrate within the biofilm ($5.22 \times 10^{-6} \text{ m}^2 \text{ h}^{-1}$). The obtained Φ value assuming that all biofilm is active was 3.21 which indicates that the process would be transport limited by the diffusion within the biofilm. The same calculation using an active biofilm thickness of 0.3 mm determined experimentally resulted in a Φ value of 1.16 which indicates that the oxygen uptake rate within the biofilm was only slightly higher than the diffusional transport of oxygen within the biofilm.

As shown by Mirpuri (1996), the fraction of toluene degrading cells within a biofilm decreases with the time of exposure and with concentration of toluene, suggesting that the distribution of toluene degrading cells was negatively correlated with the toluene concentration within the biofilm, with most of the active cells residing near the substratum. The accumulated layer then reduces the toluene concentration to which cells are exposed without producing an equivalent reduction in the transport rate (Φ close to one). The outer layer of biofilm protects the deeper layer against high concentration of toluene.

$$Bi = \frac{k_L L_b}{D_b} \quad (3)$$

To compare the importance of the mass transport within the biofilm and the mass transfer through the liquid layer (Figure 5) the Biot number was calculated using Equation 3, where k_L is the global mass transfer coefficient in the liquid layer (0.005 m h^{-1}), L_b is the total biofilm thickness (0.0023 m), and D_b is the diffusion coefficient of the substrate within the biofilm ($5.22 \times 10^{-6} \text{ m}^2 \text{ h}^{-1}$). The Bi obtained was 2.20 indicating that the transport rate for oxygen in the liquid layer was 2.2 times higher than within the biofilm, which implies that both transports are important to the overall rate of degradation. No mass transport resistance was observed at the gas-liquid interface.

CONCLUSIONS

In a *Pseudomonas putida* 54G biofilm grown in a flat plate vapor phase bioreactor,

1. The degradation process was oxygen limited for toluene gas concentration over 1.5 g m^{-3} , and the biofilm thickness ranged between 1.5 and 2.3 millimeters. The stoichiometry of the process indicated high biomass production for low toluene concentrations and high mineralization for toluene gas concentrations above 0.39 g m^{-3} .
2. Dissolved oxygen was observed to follow a linear pattern through the overlying liquid layer, indicating that mass transfer was primarily by molecular diffusion in this layer. The upper portions of the biofilm, nearest the liquid layer, also exhibited linear dissolved oxygen profiles suggesting absence of activity in this layer.
3. The respiratory activity within the biofilm increased in the basal biofilm. The greater oxygen gradients found at the basal film suggested the greater activity of the basal film which was confirmed by respirometric staining and cryosectioning for microscopy. Results highlighted zero order kinetics with a kinetic constant of $102.2 \text{ g m}^{-3} \text{ h}^{-1}$ for a toluene gas concentration of 1.5 g m^{-3} .
4. Despite the extreme thickness of an inactive layer, often accounting for as much as 87% of the biofilm, the mass transport rate through the accumulated cells was close to the respiration rate of the active biofilm (Φ close to one). This suggests that the toluene concentration profile within the biofilm is controlling the processes of injury and death of cells in the outer biofilm (higher toluene concentrations) and the respiration rate of the basal biofilm (exposed to low toluene concentrations).

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