Biodegradation of Tetrachloroethene in Batch Experiment and PHREEQC Model; Kinetic Study

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Abstract

Introduction: Bioremediation and biodegradation are considered as environmental friendly techniques for contaminants’ removal in polluted environment. In this study the removal and kinetics of Tetrachloroethene (PCE) and Trichloroethene (TCE) microbial degradation, their inhibitory effects and the rate of dehalogenation capacity at high concentration of PCE were investigated.

Materials and Methods: Dechlorinating culture was provided by Bioclear B.V. from a PCE-contaminated site (Evenblij in Hoogeveen-The Netherlands). The batch apparatuses were placed in an orbital shaker at 150 rpm at room temperature. In all the 18 batches, 6 different concentrations of PCE were measured from 0.1 mM to 0.6 mM. The degradation rate of PCE, Trichloroethene (TCE), and cis-1,2-dichloroethene (cDCE) were determined by the PHREEQC model.

Results: The results revealed that the final product was ethene and the rate of dechlorinating of PCE increased gradually. The degradation process started after 3 days in batch modes (0.1 mM). After 10 days, the dechlorination of PCE to TCE was obtained in a low concentration of PCE (0.1 mM). Also, the TCE concentration became close to zero after 10 days. However, the start point was longer than PCE and the rate of biodegradation of TCE was faster than PCE. PCE did not show any progress in the dechlorinating procedure at 13th and 14th batch series and none of the daughter products were observed.

Conclusions: It should be concluded that there was no single organism that could dechlorinate PCE to ethene, directly. Therefore, the best consortium of microorganisms to dechlorinate PCE to ethene faster, with less production of VC as the most hazardous compound, should be studied.

Keywords: Biodegradation, Tetrachloroethene, PHREEQC Model, Dechlorination


Introduction

Tetrachloroethylene (Cl₂C=CCl₂) with the systematic name tetrachloroethene or perchloroethylene (perc, PERC, or PCE) is a chlorocarbon and a colorless liquid sometimes called "dry-cleaning fluid". It is estimated that almost 85% (76.39%-99.69%) of tetrachloroethylene produced is released into the atmosphere (with a lifetime of about 2 months in the Southern Hemisphere and 5-6 months in the Northern Hemisphere); and about 10% (0.23%-23.2%) is in water, 0.06-7% is in soil and the remainder is in sediment and biota. Tetrachloroethylene is a typical soil pollutant.1-3 The PCE cleanup from groundwater is more difficult than from soil spills, because of its mobility, high toxicity even in low concentrations, and its density. As a result, the current research is focused on in-place remediation of contaminants including bioremediation and biodegradation.4,5

Degradation products observed in a laboratory include phosgene, trichloroacetyl chloride, hydrogen chloride, carbon dioxide, and carbon monoxide. The degradation products of tetrachloroethylene include trichloroethylene, dichloroethylene, vinyl chloride, ethylene, and ethane. The by-products of aerobic biodegradation of PCE include trichloroethylene, cis-1,2-dichloroethene, and vinyl chloride; while its full degradation converts it to ethene and dissolved hydrogen chloride in water.6,7

Trichloroethylene (TCE) is used for cleaning grease from industrial instruments. As an abundant environmental pollutant in groundwater, in some places, TCE undergoes reductive dechlorination catalyzed by anaerobic bacteria and produces vinyl chloride, which is a potent human carcinogen. Since air stripping or dumping methods are not currently permitted for removal of TCE, recent efforts for its removal from soil and water are focused on biological degradation and removal.8

Recent researches have focused on in-place remediation of TCE from soil and groundwater instead of disposal or...
removal using off-site treatment. Bacteria species for the degradation of TCE include anaerobic Dehalococcoides sp. and aerobic Pseudomonas fluorescens, Nitrosomonas Euroepaea and Pseudomonas putida. It is observed that in some cases *Xanthobacter autotrophicus* can produce CO and CO$_2$ from biodegradation of TCE.$^{9,10}$

The TCE spontaneous biodegradation could produce as many as four by-products: dichloroacetate, glyoxylate, formate, and carbon monoxide. The type and proportion of these by-products depend on the environmental circumstances.$^{11}$ Figure 1 illustrates the biodegradation of TCE and its by-products.$^3$

![Diagram of TCE biodegradation](image)

**Figure 1.** Aerobic (a) and Anaerobic (b) Biodegradation of TCE and its By-Products.$^3$
Both PCE and TCE are suspected carcinogens and some of the most abundant environmental pollutants of groundwater. In some groundwater, they undergo reductive dechlorination catalyzed by anaerobic bacteria that yields vinyl chloride, a potent human carcinogen. Removal by dumping or air stripping is now largely disallowed and this has focused efforts on biological methods of PCE and TCE remediation in soil and water. Aerobic TCE biodegradation pathways are found in the EAWAG-BBD.12

These man-made chemicals are very important, common, and persistent contaminants in the environment, particularly in the groundwater. Improper disposal and use of these chemicals have caused environmental concern and create significant problems for public health and environmental problems due to their toxicity and harmful effects.13-20 PCE is a nonflammable, colorless liquid and immiscible in the water and behaves more like oils; but PCE can sink to an impermeable layer in comparison to oil that floats on the surface of the water. Therefore, PCE belongs to the group of Dense Non-aqueous Phase Liquids (DNAPLs).21-23 PCE acts as a persistent contaminant and the lifetime of TCE and PCE in the atmosphere is 6 to 100 years. PCE can be dissolved in groundwater and remain for many years. In the initial step, the PCE plums are small and groundwater flows through the PCE plums and converts it into a big plume. Therefore, control of PCE is not easy since it can pollute a wide area of the environment that is difficult or maybe impossible to remediate.22 PCE is classified as Volatile Organic Compounds (VOCs). This refers to an important characteristic of PCE about the evaporation of this liquid which is very fast. The harmful property of this chemical is that it also may dissolve in sufficient amounts of water and it becomes a health concern. PCE moves easily through the soil and reaches groundwater. This property makes this chemical as a harmful one for human health due to contaminating the groundwater that is used as drinking water. There are some routes for human exposure to PCE and TCE such as drinking, swimming, food or laundring.21,22 Tetrachloroethene (PCE) possesses cytotoxicity and carcinogenicity characteristis for humans as well as animals. Tetrachloroethene (PCE) has been listed as a carcinogen agent (group 2B) by the International Agency for Research on Cancer (IARC) The United States Environmental Protection Agency (EPA) (Class B2) which indicates its probable human carcinogen. Under the Safe Drinking Water Act, the U.S. EPA has set the maximum contaminant level of PCE in groundwater at 5 μg/L.24,25 PCE exposure causes adverse health impacts. This hazardous chemical is associated with dizziness, headache, confusion, liver/kidney cancer, nervous system effects and possibly death.13,21

Bioaccumulation and persistence of PCE and its daughter dechlorinated products in the environment causes their resistance to both chemical and biological degradation. Therefore, it is highly considered for environmental pollution and human health problems.26,27 Biological degradation is an environmental friendly remediation technique to transform toxic chlorinated compounds into harmless products. Bioremediation has the advantage of chemical-physical techniques where pollutants are often transferred to another phase.29,22 Under anaerobic conditions, PCE can be biodegraded to trichloroethene (TCE), dichloroethene isomers (DCEs), vinyl chloride (VC), ethene (ETH). In the most favorable condition for PCE biodegradation, it can be completely degraded to CO₂ and H₂O. These favorable conditions that will happen in the absence of oxygen, called anaerobic conditions.30,31 The transformation of PCE to TCE occurs in a strongly anaerobic environment in the absence of oxygen, nitrate, sulfate, etc., which are more favorable to serve as electron acceptors than chlorinated solvents.30-32 A few metabolic classifications of bacteria carry out the dechlorinating process of PCE or TCE. Methanogens, sulfate-reducing, and dechlorinating bacteria are the most important ones which play role in the dechlorinating process. The behavior of these different bacteria groups can vary in many ways especially in choosing the electron donors as the source of energy for their living and growth.33-35 Based on some microbial studies, scientists found that microorganisms (e.g. methanogens, sulfate reductants, and dechlorinating bacteria) that gain their energy from the dehalogenation of chloroethene, have consumed these components as a primary substrate for their metabolism.36,37

This project aimed to investigate the kinetics of microbial degradation of PCE in a wide range of chlorinated ethenes concentrations and characterize the microbial activity by applying some simultaneous batch experiments with a combination of some electron donors and acceptors in the existence of relevant microorganisms. Also, the inhibitory effects of different chlorinated products through the dechlorination procedure, performance of batch experiments determine and quantify the dehalogenation capacity at a high concentration of PCE in a small-scale biodegradation environment which provides key biodegradation parameters such as degradation rate are the result of this study.

Materials and Methods
This study investigates the enhanced biodegradation of PCE in high concentration cultures with similar condition in source zones. The experiment has conducted in 96 days with 18 batches. The concentration will begin from 0.1mM to 0.6 mM with 3 times repetition to reduce the error of measurement as much as possible.

Chemicals
All the chemicals used in this study were in analytical grade. Liquid Tetrachloroethene (PCE) and Trichloroethene (TCE), cis-1,2-dichloroethene (cDCE) (99.9%) and trans-
1,2-Dichloroethene (tDCE) (99.9%) were purchased from Merck Company, Germany. Methanol, Na-acetate, K-L-lactate, NH₄Cl, KH₂PO₄, and NaHCO₃, and Resazurin are the ingredients of bacterial medium were provided from Sigma-Aldrich, USA.

**Bacterial Requirements for Biodegradation Process**

Dechlorinating culture was provided by Bioclear B.V. from a PCE-contaminated site (Evenblij in Hoogeveen-The Netherlands). Groundwater samples that were prepared in anaerobic conditions were delivered in green-glass bottles. To keep the anaerobic conditions, 10 ml of samples were added in 90 ml of anaerobic medium in a glove bag with 120 ml glass vials. Before using the anaerobic medium, the medium was autoclaved and contained: 2 mM Na-acetate, 5 mM K-L-lactate, 1.6 mM NH₄Cl, 0.37 mM KH₂PO₄, and 16.4 mM NaHCO₃. These combinations of nutrients have included enough elements to provide energy for growing microorganisms. Resazurin (1 mg/L) also was added as a redox indicator. To ensure that the medium is completely reduced, a few grains of Na₂S were added. Groundwater contains the bacteria was added to the medium and then the PCE dissolved in methanol (50 mM PCE) was added in the vials in the range of 0.1 to 0.6 mM. The batch apparatuses were placed in an orbital shaker at 150 rpm at room temperature (18 ºC).

**Sampling**

All concentrations of PCE are performed in triplicate. In all the 18 batches, 6 different concentrations of PCE (from 0.1 mM to 0.6 mM) were prepared. After the cultivation process, samples were collected at various time intervals to investigate the time-dependent de-chlorination. The multi-step sampling was carefully performed in order to provide an anaerobic condition for bacteria as much as possible. Firstly, 2 mL vials are labeled with the date and name of the samples. To reduce the error percentage, the experiment on the duplicate of each of the above samples was also conducted. The weight of the vials, including their caps, was determined and recorded. Afterward, 30 mL of H₃PO₄ was added to each vial to prevent further degradation of chlorinated ethane. Finally, the samples with a concentration of more than 0.1 mM of PCE were diluted with distilled water to avoid any overshoot in the GC column.

While transferring the samples into vials, following guide directions applied to make the environment as anaerobic as possible. Before the sampling, Na₂S₂ was injected into vials to ensure that anaerobic conditions were provided. The vials were instantly closed to avoid dismissing volatile compounds. To take a sample from each batch, the syringes were rinsed with methanol, and then at least twice with water, also new syringes were used for each batch of samples. Then, the samples were analyzed on a GC. The standards of PCE, TCE, tDCE, and cDCE were prepared by adding a certain amount of each compound to the serum.

**Analytical Method**

Gas chromatography (GC) is an analytical technique to separate compounds based on their volatilities. In this study, Gas chromatography (GC) was used to determine the concentration of each chlorinated product of PCE. This apparatus was equipped with two detectors, Electron Capture Detector (ECD) and Flame Ionization Detector (FID). After taking the samples, it could take a while that equilibration of water samples and headspace of 2mL vials by use of Solid Phase Micro Extraction (SPME) (Supelco) device would be achieved. SPME was inserted into the inlet of GC for 3 minutes for desorption of the adsorbed volatile compounds at 240°C. GS-GasPro column (30 mmx0.31mm) was used for chromatographic separation. In addition, helium was used as the carrier gas. The GC oven was initially set at 30 °C for 3 minutes, heated at 30 °C/min to180 °C and 25 °C/min to 230 °C, and kept at 230 °C for 10 minutes.

**Model Development**

Biodegradation rate (d<neg>ᵢᵣᵣ), which i and j are the component and bacterial consortium respectively, is so important. Batch experiments were performed to gain coefficients in this study. As an assumption, there was one bacterial consortium that can degrade PCE to ethane, completely. Therefore, the j can be dropped based on this assumption. Several models have been developed to describe anaerobic dechlorination. Michaelis-Menten model is the most common one, which prescribed a linear relation between the increases of growth rate, followed by a stationary phase with higher substrate concentrations:

\[
\frac{dC_j}{dt} = \mu_{max,i} \frac{X}{K_{m,i} + C_i}
\]

where \(\mu_{max,i}\) (1/t) is the maximum specific growth rate, X (M/L3) is the biomass concentration, C_j (M/L3) is the water phase concentration of the component, and K_{m,i} (M/L3) is the half-saturation constant. The index i is the symbol for the chloroethene (PCE (i=1), TCE (i=2), cDCE (i=3)). Another assumption for this model is the sufficient concentration of electrons that have no limit on the dechlorination process. In the batch system, where there is no addition for soil and pure phase DNAPL, the reduction equation of chlorinated components would be:

\[
\frac{dC_j}{dt} = -\mu_{max,i} \frac{X}{K_{m,i} + C_i} + \mu_{max,i} \frac{X}{K_{m,i} + C_{i-1}}
\]

The additional part in the right hand of this equation accounts for the production of TCE by PCE and DCE by TCE. If all the concentrations mention based on mol/L, so
the equation reduces to:

$$\frac{dX}{dt} = -K_{\text{decay}} X + Y \sum r_{ij}^{\text{deg}}$$

Where $K_{\text{decay}} [1/t]$ is the decay coefficient, $X$ and $Y$ are the biomass concentration and yield coefficient, respectively. $Y$ in this equation is constant. This means that the biomass concentration is gradually decreasing by the rate coefficient of $K_{\text{decay}}$. In addition, due to the biodegradation of chlorinated components with the rate of $r_{ij}^{\text{deg}}$, biomass increases. According to this assumption, the bacterial consortium could grow on all chlorinated products and the growth rate is not dependent on the DNAPL component.

**PHREEQC Model**

PHREEQC is a computer program to simulate the chemical reactions and transport in natural or polluted water. The main capability of this program is to model kinetic reactions with rate equations that are user-specified in the form of Basic Statements and to model batch reactions with user-defined expressions and calculate the concentration of elements, molarities, and activities of aqueous species. PHREEQC can model the formation and degradation of ideal, multi-component solid solutions. In this study, the coefficients that were used in the degradation equation and calculation of the degradation rate of PCE, TCE and cDCE were determined by the PHREEQC. PHREEQC model was running with some initial input data. The input data that introduced to the program were the characteristics of chloroethene and the equations and their related parameters. The main part in the input data was the $\mu_{\text{max}}$ and $K_{\text{m}}$ that had to be changed for each component. Each compound would have a unique constant. The result of the running of PHREEQC was shown in the final Fig. The coefficients for this kinetic reaction were estimated by matching the experimental data with the model results.

**Results**

Through these sequential reductive dechlorination steps, the chlorine atoms are replaced with hydrogen atoms and the concomitant production of hydrochloric acid (Figure 2).

![Figure 2. Sequential Reduction of PCE to Ethene by Anaerobic Reductive Dechlorination.](image)

Dehalogenation of tetrachloroethene takes place in batch modes with initial PCE concentrations of 0.1 to 0.6 mM. Degradation of tetrachloroethene did not carry out at higher initial PCE concentration. According to the expectation, the final product should be ethene. The results of some of the batch experiments (No. 1, 2, and 18) are shown in Figures 3, 4, and 5, respectively.

The rate of dechlorinating of PCE increased gradually with time and the daughter dechlorinated products produced more through these interactions. The degradation process started after 3 days in batch modes at low concentration (0.1 mM). This duration is considered as an adaptation time for bacteria. After almost 10 days, the dechlorination of PCE to TCE was obtained in a low concentration of PCE (0.1 mM). The time required for degradation of TCE to DCE was as long as the time required for dechlorination of PCE to TCE. As a matter of fact, the concentration of TCE in the batch mode and low concentration was almost zero after 10 days.

![Figure 3. The Results of Batch No. 1 for Degradation of TCE Experiment and PHREEQC Model.](image)
However, the start point was longer than PCE because the TCE was the product of PCE dechlorination. So, it would be concluded that the rate of biodegradation of TCE was faster than PCE.

Dehalogenation of DCE was slower than PCE and TCE. This could have caused the accumulation of this product in the cultural media.

According to the experiment, PCE did not show any progress in the dechlorinating procedure at the 13 and 14th batch series and none of the daughter products were overused. In batch no. 15, there was PCE degradation and the existence of daughter products at the same initial PCE concentration. Thus, the 13 and 14th batch series were skipped in further sampling after 49 days and would not consider in the final results.

Generally, the initial concentration which was measured was a little less than expected. It can be due to the volatility of chlorinated compounds that would disappear before the measurement time. As shown in the Figures (Figures 3-5), the concentration of cDCE, was more than the initial concentration of PCE. Whereas, the cDCE is the daughter product of PCE, the higher concentration of this product in the batch environment is impossible. It might be caused by an error in sampling.

It found that the lag time for starting the dechlorination procedure also increases when the concentration of PCE in batches increased. One reason for this phenomenon can be the toxicity effect in a higher concentration of PCE. The results of the kinetic model are shown in Table 1. According to the results, the result of estimated constants in the model was in good agreement with the results obtained from the experiments.

**Table 1. The Results of the Kinetic Model**

<table>
<thead>
<tr>
<th>Compound</th>
<th>PCE</th>
<th>TCE</th>
<th>cDCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{max}$ (1/h)</td>
<td>7E-10</td>
<td>5E-10</td>
<td>9E-11</td>
</tr>
<tr>
<td>$K_{on}$ (M)</td>
<td>9E-05</td>
<td>2E-5</td>
<td>5E-11</td>
</tr>
<tr>
<td>Biomass, X</td>
<td>1.4E-03</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

2 obtained from data fitting of batch experiments and PHREEQC model

**Discussion**

Determination of the sequential reductive dechlorination steps can help to understand the mechanism of the degradation of halocarbon compounds. As can be seen in Figure 1, the hydrogen can act as an electron donor and the chlorine atoms are replaced with hydrogen atoms and the concomitant production of hydrochloric acid. On the other hand, some by-products that are more toxic for the environment and human health could be produced in this
cycle. The cDCE and VS are the most toxic by-product during the degradations. However, the ethene as a final product is known as a harmless compound that would be generated.

Generally, batch studies are performed to determine the potential of PCE dehalogenation at high concentrations through biological processes.\textsuperscript{45} Dechlorination of PCE to ETH using H\textsubscript{2} or ethanol as an electron donor would be possible in the consortium of PCE/CH\textsubscript{3}OH, PCE concentration, available oxygen, and partial pressure of H\textsubscript{2} are the most important factors in the extent of dechlorination of PCE. In a high concentration of PCE, the dechlorination rate is extended, subsequently.

Based on the results, ETH formation from VC in absence of PCE was faster than in the presence of PCE. It is found that the degradation rate will be 3 times more when the molecular hydrogen was supplied as an electron donor instead of methanol.\textsuperscript{46} It means that H\textsubscript{2} is the primary electron donor for reductive dechlorination of PCE in a methanol/PCE fed culture.\textsuperscript{47} Therefore, PCE degradation to ETH could be possible in this enriched consortium, since VC cannot accumulate more than 50% of PCE initial concentration.

DCE accumulation increases with increasing the PCE concentration. The high concentration of DCE might result from PCE toxicity to DCE and VC dechlorination, DCE toxicity to DCE and VC dechlorination, slow DCE dechlorination kinetics, and competition between different dechlorination steps for electron donor.\textsuperscript{48} This is a sign for the removal of PCE DNAPL through the dehalogenation procedure. Since DCE accumulation means that PCE dechlorination was started.

In the subsurface of the environment, there are lots of strains of bacteria that can reduce PCE and TCE to c-DCE. However, the bacteria which also dechlorinate cis-DCE and VC to ethene are more limited. So, cDCE was the predominant product and tDCE was less observed in groundwater. The degradation of all components is not as fast with the increasing concentration of PCE. There are some inhibitory effects of dechlorinated products of PCE throughout the whole process. The inhibitory factors cause to decrease in the rate of degradation and microbial growth.

Inhibition is a competitive phenomenon in which the more chlorinated compounds inhibited reductive dechlorination of the less chlorinated compounds. It means that PCE inhibited reductive TCE dechlorination, but not DCE and VC, while TCE inhibited DCE and VC dechlorination and DCE inhibited VC transformation to ethane.\textsuperscript{46} It was reported that in all PCE concentrations, ethene production occurred after the transformation of most of DCE to VC. Therefore, this result indicates that DCE inhibits strongly VC dechlorination to ethane.\textsuperscript{44} However, the production of ethene has no inhibitory effects on the dechlorination of PCE in any concentration. On the other hand, the rate of dechlorination increases in the existence of more ethene concentrations in the culture. This fact can be explained as the inhibitory effect of ethene concentration on methanogenesis and reduce the activity of methanogens. Therefore, one of the competitors for the substrate was almost omitted and more substrate was available for dechlorinators for dehalogenation reactions.\textsuperscript{47,48} Also, it was reported that chloroethene degradation under aerobic oxidation can happen at a redox conditions range which means that reducing environments is required for ethene compounds degradation.\textsuperscript{10}

In addition, when TCE initial concentration increased, VC accumulation decreased and the ethene production rate increased.\textsuperscript{49} However, VC production does not affect TCE dechlorination. So, it reveals that the VC accumulation that occurred in PCE dechlorination should not inhibit the activities in the last step of the dechlorination procedure. On the other hand, it was detected the VC and ethene (as donors of the electron at aerobic chloroethene oxidation) at high concentrations can accelerate the growth of ethenotrophs and degradation of compounds.\textsuperscript{10,50}

It found that the lag time for starting the dechlorination procedure also increases when the concentration of PCE in batches increased. One reason for this phenomenon can be the toxicity effect in a higher concentration of PCE. Also, greater time of exposure to high concentration, the effects of direct contact with DNAPL and more needed time for adapting the microorganisms in the more toxic environment were the other possible reasons for lag time differences in a high concentration of PCE.\textsuperscript{45} The most important inhibitory effect could be explained as the toxicity of PCE in high concentrations for microorganisms. In this phenomenon, the dechlorination process might be inhibited or performed with a long lag phase. As it is shown
in figs with 0.6 mM concentration of PCE, no degradation of PCE occurred after 96 days of starting this experiment. The model could not represent the observed concentration in the higher initial PCE concentrations, where no degradation was taken place. Therefore, one other assumption in the model was that the degradation was independent of the initial PCE concentration.

**Conclusion**

In this study, the microbial kinetics of the anaerobic degradation of PCE was investigated. Batch experiments were carried out to characterize the microbial activities and the maximum rates of utilization or formation were estimated. At the end of the batch experiments, the percentage of reduction can be measured by levels of dechlorination products formed and the electron donors consumed.

Bioremediation was considered as one of the most effective tools for the degradation of PCE to harmless products. PCE mainly dechlorinated to ethene, and intermediate dechlorinated products were TCE, DCE, and VC. Through the production of dechlorinated products, VC accumulates. The main cause of the accumulation of VC was the rate of degradation, which was the slowest through the dechlorinating process. Therefore, the main concern was related to VC accumulation in contaminated groundwater, since this substance is the most toxic contaminant among the other chlorinated products. There was some microbiological evidence that concludes that there was no single organism that could dechlorinate PCE to ethene, directly. In this regard further studies are needed to find the best consortium of microorganisms to dechlorinate PCE to ethene faster, with less production of VC as the most hazardous compound through the dechlorination process.

**Authors’ Contributions**

SSM have taken part in conducting of the study and AHH intellectual helping in different stages of the study is appreciated. AA got involved in data analysis and manuscript preparation. NA has done technical analysis and manuscript preparation.

**Conflict of Interest Disclosures**

The authors declare that they have no conflicts of interest.

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