FINAL REPORT-PHASE I

Application of Biofilm Covered Activated Carbon Particles as a Microbial Inoculum Delivery System for Enhanced Bioaugmentation of PCBs in Contaminated Sediment

SERDP Project ER-2135

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List of Acronyms

PCBs	Polychlorinated biphenyls
A1248	Aroclor 1248
DHPLC	Denaturing high performance liquid chromatography
EPS	Extracellular Polymeric Substances
GAC	Granular Activated Carbon
PNA-FISH	Peptide nucleic acid Fluorescence *In situ* hybridization
SEM	Scanning Electron Microscopy
CLSM	Confocal Laser Scanning microscopy
DAPI	4',6-diamidino-2-phenylindole (fluorescent stain)
Q-PCR	Quantitative Polymerase Chain Reaction

Keywords

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- Mr. Marshall Phillips, Back River Wastewater Treatment Plant, Baltimore for providing the sludge samples.

1. Abstract

Objectives: The objectives in SERDP project ER-2135 addressed the SERDP Exploratory Development (SEED) Statement of Need ERSEED-11-01: *In situ remediation of contaminated aquatic sediments*. Removal of the class of persistent organic pollutants, polychlorinated biphenyls (PCBs), from contaminated aquatic sediments is a priority due to their ability to enter the food chain and their potent toxic and carcinogenic properties. Presently, the approved remediation methods mainly include dredging and capping. However, these techniques are not only expensive, but also result in increased PCB concentrations in the water phase due to resuspension of contaminated sediment particles. While *in situ* microbial degradation of PCBs would represent a significant improvement in remediation efforts, previous attempts have failed due to PCB stability, low bioavailability and the low abundance and activity of naturally occurring PCB-degrading microorganisms. In order to overcome these negative aspects of microbial degradation, this project evaluated an approach, where anaerobically dechlorinating biofilms were added to sediment as a delivery system either by utilizing bacteria localized and concentrated onto activated carbon surfaces in active biofilm communities or by applying enriched wastewater sludge biofilms. The enhanced effect of biofilms on bioaugmentation was examined in a subsequent mesocosm experiment, where these biofilm communities were applied to PCB contaminated sediment.

The high efficiency of activated carbon to quickly adsorb and sequester PCBs from aquatic sediments has previously been demonstrated. Co-localizing PCB-degrading microbes onto the surfaces of activated carbon in the form of biofilms and utilizing it as a microbial inoculum delivery system provides a number of benefits. First, the sequestering capacity of activated carbon further lowers aqueous concentration of PCBs that have leached from sediment. Second, by providing a large population of PCB-degrading microbes directly adjacent to sequestered PCBs, the degradation capacity and ability of the adherent microbial populations are augmented. These close spatial relationships are required for microbes to utilize PCB as an electron acceptor and enable subsequent degradation. Third, since microbes are embedded within an adherent biofilm, they can be applied to aquatic environments and maintained in high numbers without being washed away in the fluvial system. The application of the enriched sludge biofilm system is an alternative approach, where the biofilm has already formed on the organic backbone of the sludge comparable to the activated carbon particles.

The proofs of concept objectives in this project were: 1) To evaluate if and how biofilms (made up by dense populations of dechlorinating bacteria) associated with a surface can be utilized to obtain enhanced microbial PCB degradation in aquatic sediments; 2) To examine how the delivery system consisting of an active microbial biofilm attached to a surface will influence PCB dechlorination rates and extent as well as PCB dechlorinating biofilm populations in mesocosms, when they are used as a delivery system for bioaugmentation.

Technical Approach: Biofilm cultures were grown anaerobically for DF1 and the enriched wastewater sludge biofilm and aerobically for the *Burkholderia xenovorans* LB400 biofilm. Active DF1 and *B. xenovorans* cultures were mixed with 3% activated carbon and used as a delivery system together with the enriched wastewater sludge biofilm. All biofilm samples were delivered and mixed into sediment from Grasse River, NY with approximately $2 \cdot 10^6$ cells/g of

sediment inoculum. Autoclaved sediment was applied as control. A final concentration of 50 ppm A1248 was applied in order to obtain relatively fast dechlorination rates that would be possible to evaluate within the project period. Samples were collected in triplicate at day 0, 28, 60, 80, 135, 160 and 200. The following methods were applied: Extraction of DNA and PCBs from sediment followed by PCBs analysis with a standard gas chromatography method. Extracted DNA was analyzed via PCR and DHPLC for bacterial diversity and with qPCR for the abundance of dechlorinating bacteria. Several microscopy methods were applied to evaluate the biofilm: DAPI, SybrGreen/CLSM, SEM (microscopy and elemental analysis) and conventional FISH and PNA-FISH.

Results: Anaerobic growth of the dechlorinating bacterium DF1 was observed on activated carbon particles via multiple staining and microscopic techniques. This is the first time that biofilms made up by this organism have been confirmed and subsequently visualized by the applied techniques. In addition, enriched dechlorinating biofilms originating from wastewater sludge were also examined. The sludge biofilm contained six to seven different dechlorinating phylotypes and a high abundance of dechlorinating bacteria that were mixed with other indigenous wastewater organisms. The analysis by staining and microscopy made it possible to confirm the presence of biofilm on the activated carbon particles as well as the structure of the enriched sludge biofilm.

The dechlorinating biofilm covering activated carbon particles or located in the enriched sludge biofilm were applied as microbial inoculum systems to mesocosms consisting of PCB contaminated sediment from Grasse River, NY. The results showed that the dechlorinating bacteria remained in the sediment throughout the experiment and increased almost 2-fold in numbers. No difference was seems between the abundance between the two types of inoculum. Both types of biofilm inoculum enhanced dechlorination of PCBs in the sediment mesocosms compared to untreated sediment as well as liquid cultures of the anaerobic dechlorinating bacteria. This was shown when calculating the dechlorination as decrease in chlorines per biphenyl and in the dechlorination rate (mol%) over 200 days. Examination of the dechlorination of the individual PCB congeners for the mesocosm augmented with the two types of biofilm inoculum showed that the dechlorination caused by the biofilm covered activated carbon particles with DF1 was less extensive compared to the enriched sludge biofilm. The reason was the high diversity of dechlorinating organisms in the sludge that made more extensive dechlorination of the congeners in Aroclor 1248 possible compared to the specific activity by DF1 (flanked *meta* or *para* dechlorination). These results show that the proof of concept documented.

Benefits: Application of either of the two biofilm based inoculation systems enhances dechlorination of PCBs in sediment and thus makes the congeners available for subsequent aerobic degradation and removal as contaminant from the environment. This is obtained due to the simultaneous adsorption of PCBs onto the organic surface that the biofilm is attached to, the large number of PCB dechlorinating organisms located directly adjacent to the adsorbed PCB and the benefit of the bacteria being embedded within an adherent biofilm that protects them from being environmental stressors and from being washed away in the fluvial sediment system.

2. Objective

The objectives in SERDP project ER-2135 addressed the SERDP Exploratory Development (SEED) Statement of Need ERSEED-11-01: *In situ remediation of contaminated aquatic sediments*. Removal of the class of persistent organic pollutants, polychlorinated biphenyls (PCBs), from contaminated aquatic sediments is a priority due to their ability to enter the food chain and their potent toxic and carcinogenic properties. Presently, the approved remediation methods mainly include dredging and capping. However, these techniques are not only expensive, but also result in increased PCB concentrations in the water phase due to resuspension of contaminated sediment particles. While *in situ* microbial degradation of PCBs would represent a significant improvement in remediation efforts, previous attempts have failed due to PCB stability, low bioavailability, and the low abundance and activity of naturally occurring PCB-degrading microorganisms. In order to overcome these negative aspects of microbial degradation, this project evaluated an approach, where anaerobically dechlorinating bacteria were localized and concentrated onto activated carbon surfaces in active biofilm communities. The enhanced effect of on bioaugmentation was examined in a subsequent mesocosm experiment, where these biofilm communities were applied to PCB contaminated sediment.

The high efficiency of activated carbon to quickly adsorb and sequester PCBs from aquatic sediments has previously been demonstrated. Co-localizing PCB-degrading microbes onto the surfaces of activated carbon in the form of biofilms and utilizing it as a microbial inoculum delivery system provides a number of benefits. First, the sequestering capacity of activated carbon further lowers aqueous concentration of PCBs that have leached from sediment. Second, by providing a large population of PCB-degrading microbes directly adjacent to sequestered PCBs, the degradation capacity and ability of the adherent microbial populations are augmented. These close spatial relationships are required for microbes to utilize PCB as an electron acceptor and enable subsequent degradation. Third, since microbes are embedded within an adherent biofilm, they can be applied to aquatic environments and maintained in high numbers without being washed away in the fluvial system. The application of the enriched sludge biofilm system is an alternative approach, where the biofilm has already formed on the organic backbone of the sludge comparable to the activated carbon particles.

The high priority needs that were addressed in this proposal were: 1) To demonstrate that biofilms (made up by dense populations of PCB degrading bacteria) associated with a surface could be utilized to obtain enhanced microbial PCB degradation, and 2) To develop a delivery system with an active microbial biofilm inoculum that would sequester PCBs from the surrounding aqueous sedimentary environment as well as enhance PCB degradation.

<u>2.1 Proof of concept objectives</u>

The proofs of concept objectives in this project were:

1) To evaluate if and how biofilms (made up by dense populations of dechlorinating bacteria) associated with a surface can be utilized to obtain enhanced microbial PCB degradation in aquatic sediments
2) To examine how the delivery system consisting of an active microbial biofilm attached to a surface will influence PCB dechlorination rates and extent as well as PCB

dechlorinating biofilm populations in mesocosms, when they are used as a delivery system for bioaugmentation.

The intent of the present project was to demonstrate enhanced dechlorination by the use of biofilm covered activated carbon particles as a delivery system. An alternative biofilm delivery system (sludge based enriched biofilm) was also tested even though it was not proposed in the original proposal. Full PCB degradation requires two subsequent steps: first anaerobic microbial dechlorination to a biphenyl structure with four or less chlorines, followed by an aerobic step where the biphenyl rings are broken, thereby enabling mineralization and thus complete PCB degradation. The anaerobic process is the rate limiting step, since only few bacterial phylotypes are capable of performing reductive dechlorination, whereas bacteria capable of performing the aerobic step are ubiquitous. In addition, the abundance and activity of anaerobic dechlorinating bacteria *in situ* is low. While full degradation of PCBs is a long-term goal of this research, it was beyond the scope of this project that focused on the first and rate-limiting step of the initial anaerobic dechlorination.

It was expected that in the current study application of the delivery method would be able to show increased reductive dechlorination of extensively chlorinated congeners (dominant in Aroclor mixtures). However, due to the short time (project period of one year) it might not be possible to reach the optimal level of tetra or less chlorinated PCB congeners.

2.2 Success criteria for this SEED project

1. To establish inoculum consisting of anaerobically dechlorinating biofilms that can be used as a microbial inoculum delivery system in sediment. Two methods were examined: biofilm covered activated carbon particles and enriched wastewater sludge biofilms;
2. To develop tools that can be used to analyze biofilms formed on a surface with regards to bacterial numbers and populations as well as PCB dechlorination activity;
3. To show how a biofilm based delivery system can enhance PCB dechlorination rates and extent as well as influence PCB dechlorinating biofilm populations in mesocosms, when the biofilms are used as a delivery system for bioaugmentation.

2.3 Risk reduction/acquisition of data for potential new proposal

The goal of performing the experiments in this project was to evaluate whether biofilm formation on activated carbon particles would take place and whether the changed mode of growth in this biofilm form would promote the dechlorination activity of PCBs in sediment mesocosms. Prior to this project biofilms had not been considered as a way of growing bacteria in the laboratory for PCB dechlorination and even less considered as a method for promoting enhanced activity based on their natural mode of growth, while performing bioremediation actions in sediment. There are many benefits for bacteria to grow in biofilms and it was the goal to find out if these benefits would translate into increased activity and potentially also a more beneficial kind of activity, where the dechlorination process would not end up in a “dead-end” situation or would even be more prone to subsequent aerobic PCB degradation processes, when present in a real sediment environment and not only in laboratory test tubes.

The data obtained in this project showed that biofilm formation on activated carbon particles (1) or anaerobic biofilm originating from enriched anaerobic sludge (2) enhanced dechlorination of

PCBs in the sediment mesocosms and would be essential for developing a complete proposal for a more extensive follow-up project. Since this project contained an initial evaluation of the concept, there is a need for this evaluation to be expanded in addition to being revised due to the experiences encountered during the project.

3. Background

Degradation of persistent organic contaminants such as Polychlorinated Biphenyls (PCBs) occurring under anaerobic conditions in sediments is a critical process for the complete transformation to non-toxic forms. In sediment and soil *in situ* microbial degradation of PCBs under anaerobic conditions is a slow process due to the chemical and biological stability of the compounds, the low bioavailable concentrations and in many cases the low abundance, diversity and activity of naturally occurring PCB degrading microorganisms (Fagervold, *et al.*, 2005). Therefore, it has been suggested that *in situ* biological transformation of PCBs in sediments will not reduce the concentration sufficiently within a reasonable time frame. Based on this, impacted sites have been dredged resulting in removal to contained locations such as landfills or capped to keep the contaminants from entering the aqueous phase (Wakeman & Themelis, 2001). However, the removal or capping of impacted sediments can cause unwanted release of PCBs into the environment and the proposed risk reduction goals are often not achieved (Megasites, 2007). The physical disturbance due to dredging or capping will impact the benthic organisms directly and the concentration of PCBs in the water phase will increase due to re-suspension of sediment particles containing PCBs. This will cause harm for benthic organisms and the surrounding environment since the contaminated sediment particles will be spread.

Recent work with PCB contaminated sediments has used activated carbon to control *in situ* bioavailability of PCBs, with large reduction in the bioaccumulation of PCBs by clams, worms, and amphipods in field studies with sediment treated using 2.0-3.2% by weight granular activated carbon (Cho, *et al.*, 2009). Sediment treated with granular activated carbon attains aqueous equilibrium PCB concentrations 85% and 92% lower than untreated sediment in one-month and six-month contact experiments, respectively (Zimmerman, *et al.*, 2004). Activated carbon effectively outcompetes solid phases and benthic organisms for PCBs, resulting in lower PCB levels in the aqueous phase and reduced PCB exposure to aquatic organisms, including microbes. In addition, since the activated carbon is mixed with the sediment (injection or tilling) the particles cannot be distinguished from sooth, black carbon and other organic particles that are naturally present in the sediment. Natural *in situ* reduction of PCBs through anaerobic dechlorination has been demonstrated (Bedard & Quensen, 1995), but has not been widely adopted as a remedial alternative due to the slow and uncertain process using liquid cultures for bioaugmentation and continual exposure to the sediment ecosystem during the long attenuation period. A previous SERDP funded study (ER1502: "Application of Tools to Measure PCB Microbial Dechlorination and Flux into Water During In-Situ Treatment of Sediments") assessed how natural PCB dechlorination activity in sediments was affected by the addition of activated carbon. The results showed that indigenous PCB dechlorinating bacteria were capable of PCB dechlorination even in the presence of a strong sorbent like activated carbon. Therefore, a bioremediation solution based on a combination of activated carbon together with dechlorinating bacteria would be possible.

Complete microbial degradation of PCBs requires anaerobic reductive dechlorination of extensively chlorinated congeners, such as those present in commercial Aroclor mixtures, followed by subsequent aerobic cleavage of the biphenyl ring and mineralization of the less extensively chlorinated congeners (Field & Sierra-Alvarez, 2008, Pieper & Seeger, 2008). Anaerobic bacteria within the bacterial group *Chloroflexi* have been confirmed to have PCB dechlorinating activity *(Fennell, et al., 2004)* (May, *et al.*, 2006) (Bedard, *et al.*, 2006). However, since most of these organisms are found in low numbers and with low *in situ* activity in the environment another approach is needed in order to obtain biodegradation of PCBs that can reach proposed risk reduction goals at PCB contaminated sites. Bioaugmentation of PCBs in sediment and soils has been examined in several studies. However, the dechlorination rates and the extent of PCB dechlorination obtained with bioaugmentation were low (Krumins, *et al.*, 2009) (May, *et al.*, 2008).

A combination of sequestration with activated carbon together with bioaugmentation with liquid cultures has been examined (Payne, *et al.*, 2011). Results from this study applying dry activated carbon together with liquid cultures of anaerobically dechlorinating bacteria showed that dechlorination can occur. However, the study did not include results on whether the positive effect on dechlorination could be maintained in the long term since the inoculum was supplied as liquid culture without any supporting material.

3.1 Biofilm based delivery system

The novel two-phased biofilm approach (utilizing granular activated carbon as an attachment and growth surface for biofilm formation by PCB degrading bacteria) that was examined in this project has not been used for *in situ* bioremediation of PCBs largely because a number of factors such as predation, competition and sorption conspire against traditional bioaugmentation using liquid inoculum in non-confined systems. Also, the biofilm mode of growth of bacteria capable of degrading PCBs has not been recognized as an advantage for *in situ* bioaugmentation even though growth in biofilms is the preferred mode of growth for bacteria in the environment (Costerton, *et al.*, 1978).

Organic surfaces (such as activated carbon) have inherently high affinities for simultaneous attraction of PCB degrading biofilm forming bacteria and adsorption of PCBs ensuring close proximity to needed electron acceptors. Both processes are requisite components for the implementation of a two-phased approach, where organic compounds are applied as substratum for biofilm formation and subsequent delivery systems for bioaugmentation of PCBs in contaminated sediment. In addition, because PCB dechlorinating bacteria are hydrophobic, organic surfaces such as activated carbon can be used to effectively concentrate microorganisms from liquid cultures by surface adsorption. The organic surface with adsorbed microbial catalysts can then be used directly as inoculum in PCB contaminated sediments.

This biofilm based delivery system was therefore taking advantage of bacteria being able to adhere to and grow at a high density at the activated carbon surface, while simultaneously sequestering PCBs on the activated charcoal. The biofilm community of microbes had a larger cell density and activity than would be obtained in free floating systems, where the chances would be rare for direct interaction between PCBs and the bacteria. This interaction is required for electron transfer and subsequent PCB degradation. The dechlorinating bacteria were also fixed to the surface of the activated carbon particles, so they were not washed away or consumed by other microorganisms in the environment increasing the potential for successful long term bioaugmentation. This two-phased approach can provide a both efficient and cost effective method for inoculation of microorganisms for bioaugmentation.

Rationale for adding experiments based on enriched anaerobic sludge biofilm to this project
In addition to evaluating the effect of anaerobic biofilms formed on activated carbon particles, the effect of enriched biofilm cultures from wastewater sludge was also examined. This part was not a part of the original proposal.

The rationale for expanding the project to include anaerobic biofilms originating from sludge was results that were obtained during the project period that showed a surprising abundance of dechlorinating bacteria in the wastewater treatment plant that was investigated. Considering that the structure of wastewater sludge is similar to the delivery vehicle based on activated carbon proposed in this project it would be relevant to examine the dechlorination activity of this type of biofilm as well.

The wastewater sludge flocs mimic the biofilm mode of growth since the sludge bacteria are attached to a backbone of the floc consisting of organic and inorganic fibers and particles in addition to the biofilm itself made up of a diverse microbial population (Eighmy, *et al.*, 1983). Microscopic analysis using fluorescence in situ hybridization of the sludge flocs also revealed presence of dechlorinating bacteria (Figures 19). Thus this biofilm system capable of anaerobic dechlorination had the potential for application in the mesocosm experiments as a delivery vehicle. The principal structure of the enriched sludge delivery system is shown in figure 1.

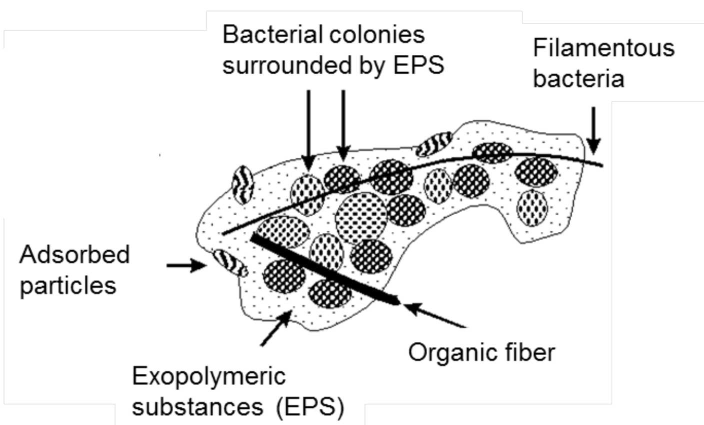

Figure 1. The principal structure of a sludge biofilm floc (Kjellerup, et al., 2001). The composition of the sludge biofilm floc and the function is similar to the biofilm attached to the activated carbon particles and would therefore make an efficient delivery system, since the dechlorinating bacteria are already present in this system.

4. Materials and Methods

4.1 Growth of anaerobic cultures for mesocosm inoculum

Anaerobic based biofilm inoculum was developed on AC particles by the use of the dechlorinating bacterium DF1 in co-culture with *Desulfovibrio* and PCB-61 at 30°C in teflon capped serum vials to maintain anaerobic conditions. 10 ml anaerobic medium (Berkaw, *et al.*, 1996) containing 10 mM formate as electron donor, 1 ml of cell extract from active cultures of *Desulfovibrio sp*., 0.5 mM titanium(III) nitrilotriacetate (TiNTA) was applied as a reductant together with 10 microliter of PCB-61 dissolved in acetone (final concentration 10 ppm) and inoculated with 2-4 ml DF-1 culture transferred from an already active DF1 culture. In inoculum containing AC, AC was added when the cultures had reached approximately 25-30 mol% of the dechlorination product PCB-23. The bacterial cells would at this stage attach to the added AC particles (3% weight), colonize and develop a biofilm on the AC particle surface, which was monitored by SEM and q-PCR. The co-culture with *Desulfovibrio* was applied, since more robust growth of DF1 has been observed due to the fact that these two organisms originally were enriched together from sediment. Cultures equaling a total of 265 mL were used for mesocosm inoculum and were started from previous DF1 cultures that were in stationary phase from polychlorinated ethene (PCE) cultures. A concentration of 3% granular activated carbon (GAC; Calgon Carbon Corporation; type46; mesh 50x200; lot no P-21553) was added to half of the cultures on day 57, when approximately 45% dechlorination had been achieved, to facilitate biofilm formation.

The enriched sludge biofilms were added to sediment mesocosm via 1 ml of enriched culture that would equal the number of dechlorinating bacteria added in the mesocosm with liquid or biofilm DF1 cultures. The enrichment of the anaerobic sludge culture was performed with 50 ppm PCB-116 (2,3,4,5,6-PCB) ensuring that dechlorination activities related to any specific position at the chlorine ring would be presented. The autoclaved sludge control was added in order to evaluate the effect of the nutrient present in the activated sludge, since the samples were taken from a nutrient (organic matter, nitrogen) removing wastewater treatment plant, where organic matter and nitrogen is accumulated in the sludge biomass.

Samples were collected anaerobically from all cultures on D0, D35, D56 and D70 to evaluate the dechlorination rate and the number of dechlorinating bacteria. Extracted DNA was verified by PCR and subjected to quantitative PCR (described below). Extracted PCBs were analyzed via gas chromatography (GC). Metabolic activity was measured by mol% PCB23 according to the total amount of PCB61 degraded to PCB23. Previous results have indicated that optimal dechlorination activity was occurring when the cultures had reached 40-50 mol% PCB23. Cultures were added as inoculum to Grass River sediment mesocosms on day 73 either as liquid DF1 cultures or as biofilm cultures formed on the activated carbon surfaces.

4.2 Biofilm formation on activated carbon particles

To verify the presence of biofilms formed on activated carbon particles prior to the setup of sediment mesocosms, anaerobic cultures of DF1 (or aerobic cultures of LB400) and activated carbon were mixed thoroughly and sampled for imaging at ICAL at Montana State University (Sub-contractor) and Center of Biofilm Engineering (CBE, Betsy Pitts) (Collaboration, not in the original proposal). Cultures were shipped over night in anaerobic vials (DF1) or aerobically

(LB400) and were imaged the next day. For the SEM (Scanning Electron Microscopy) drops of sample were air dried onto silicon wafers and coated according to standard protocols (ICAL, Laura Kellerman). Images obtained via CBE were acquired directly from the wet activated carbon samples, where the DF1 bacteria had formed biofilms. The samples were directly labeled with the fluorescent stain SybrGreen that target all DNA, but does not stain the background such as the activated carbon particles or components in the growth media.

PNA-FISH (Peptide nucleic acid-Fluorescence *In situ* hybridization) was also applied to assess the formation of dechlorinating biofilm on the surface of the activated carbon particles. The fixation, hybridization and imaging was performed according to the manufacturer's instructions (AdvanDx).

<u>4.3 Mesocosm sediment</u>

Sediment was collected and homogenized using a coring device to collect the top 5 cm of the sediment (Dr. Upal Ghosh Laboratory, UMBC). Sediment were filled into glass tanks ensuring that anaerobic layers of sediment would be present over layered with 1-2 inches of water from the sample as the sediment settled out after mixing with inoculum or appropriate controls. All preparations were performed in an anaerobic glove box.

<u>4.4 Mesocosm setup</u>

Anaerobic cultures of DF1 with and without biofilm covered GAC were centrifuged at 12,000rpm for 10 minutes to obtain an aggregate pellet which was then resuspended in 10mL supernatant per mesocosm and added to respective mesocosms at approximately $2x10^6$ cells/g of sediment. Aroclor1248 (A1248) as well as PCB 116 (2,3,4,5,6-PCB) were applied to respective experimental and control mesocosms to a final concentration of 50 ppm. Activated carbon was added to a control in a 3% concentration and was mixed into the sediment. A control for non-biological transformation was made by autoclaving sediment for 45 minutes at 121°C on the liquid setting and subsequently adding A1248 (85uL). Controls for the priming effect of culture media were made by adding the same amount of sterile (0.22um) filtered spent media as culture to sediment. The experimental setup is shown in Table 1.

Mesocosm tanks were covered with glass lids to avoid evaporation and kept in the dark at room temperature (approx. 22C). Sampling occurred after 0, 28, 60, 135, 180 and 200 days. Both PCB and biofilm specific analyses were be performed. The sampling was performed in triplicate by using inverted glass Pasteur pipettes and organized according to a grid pattern to avoid sampling from the same locations every time thus obtaining representative samples. Triplicate 1mL aliquots were taken for PCB extraction and subsequent analysis by GC in addition to DNA extraction and analysis by PCR and qPCR.

Table 1. Experimental setup for the Grasse River sediment mesocosm experiment evaluation the effect of biofilm covered activated carbon particles as a microbial delivery system.

Mesocosm Experiment	**Inoculum**	**GAC**
Anaerobic DF1 Biofilm		
Sediment primed w/ DF1 sterile spent media	None	No
Sediment inoculated w/ DF1 liquid culture	10^6 cells/ml liquid culture	No
Sediment w/ DF1 Biofilm formed on GAC	10^6 cells/ml biofilm culture	Yes
Anaerobic biofilm enriched from sludge		
Sediment spiked with ACT sludge enrich	10^6 cells/ml enriched culture	No
Sediment spiked with autoclaved activated sludge	None	No
Aerobic LB400 Biofilm		
Sediment primed w/ LB400 sterile spent media	None	No
Sediment inoculated w/ LB400 liquid culture	10^6 cells/ml liquid culture	No
Sediment w/ DF1 Biofilm formed on GAC	10^6 cells/ml biofilm culture	Yes
Controls		
Sediment (Positive control)	None	No
Autoclaved sediment (Negative control)	None	No

<u>4.5 DNA Extraction and Quantitative PCR</u>

All mesocosm samples were extracted for DNA following manufacturer's instructions (MoBio Laboratories, PowerSoil® DNA Isolation Kit). To monitor growth of mesocosm inoculum, DNA was extracted from samples of pure culture using Bio-Rad InstaGene™ Matrix. DNA was homogenized at room temperature before being sampled for PCR using Fermentas DreamTaq™ Green PCR Master Mix and 16S chromosomal specific 348F-884R primers to amplify total putative dechlorinating Chloroflexi. Aliquots of DNA applied for qPCR analysis. Bio-Rad iQ™ SYBR® Green Supermix was used along with the same PCR primers and 1µL template DNA in 25µL reactions. An MJ Research PTC-200 thermocycler was used (15min at 95°C, then 35 cyles of 95°C for 30sec, T_a=61°C for 30sec, 72°C for 30sec, then 2min at 72°C followed by a melt curve analysis from 60-95°C). Purified PCR products of the same primers were quantified via NanoDrop® 100 and used in a series of six 10x dilutions for qPCR standards. OpticonMonitor 3 software was used to obtain values for amount of DNA produced by PCR (ng), which was then converted to 16S copy numbers based on fragment length (536nts) and the average MW of a base pair (650 daltons).

<u>4.6 PCB Extraction and Gas Chromatography Analysis</u>

For every sampling date, 1 ml aliquots of either DF1 culture or 1 g of mesocosm sediment were collected (in triplicate) with sterile Pasteur pipets into PCB clean glass culture tubes. The sediment sampling followed a grid pattern to ensure that the sampling would take place in independent areas at the different time points to increase the reproducibility of the results. To extract PCBs from a given sample, 5 ml of hexane was added to the sample and shaken (3 h for pure culture and 12 h for mesocosm sediment). Just before extraction, samples with hexane were vortexed for 5 s. The hexane phase (5 ml) was after shaking transferred to an extraction column containing copper/flourisil (1:4 mix) and sodium sulfate for. The purpose of the sodium sulfate was to remove any droplets of water in the extract, whereas the Cu/fluorisil removed sulphide from the anaerobic processes and protein from the organic matter and cell material present that might interfere with the identification of congeners in the GC analysis. 1 ml of cleaned extract was transferred to a GC vial, where the internal standards (PCB30 and PCB204) were added to a final concentration of 10 ppm. Standards were purchased from commercial vendors and were traceable to NIST standards. Each extract was analyzed for PCB congeners using an Agilent Technologies gas chromatograph (GC) equipped with a split/splitless injector, a 60 m DB-5 capillary column, and an electron capture detector. Helium and Argon/Methane were used as carrier and make-up gases and the GC oven was programmed from 70 to 300°C over 110 minutes. The chromatographic resolution was comparable to EPA standard methods. PCB congeners were identified by comparing their chromatographic retention times and relative responses to authentic PCB congener standards containing all congeners detected in Aroclor mixtures according to the EPA (referred to as Mullin's Mix). Additional congeners were added to the standard Mullins Mix in order to account for the dechlorination products that were formed due to microbial activity. In total, 173 congeners were identified in the GC method and the majority of these eluted off as single congeners. In cases where two or more congeners co-eluted, the amount was assumed to be equal for the congeners and split accordingly. To assure the quality of the GC analysis and results the following actions were performed: 1) blanks consisting of hexane were run before, during and after each sample sequence to ensure low and consistent blanks; 2) Surrogates were added to samples to ensure reproducible recoveries (non-Aroclor PCB congeners (14, 65,166). Due to the fact that calculations were performed based on molar percent, the results were not adjusted for recovery different from 100%. The quality objective for the surrogate recoveries is >70%, with a 25% relative standard deviation. The quality objective for accuracy is 20% of the certified values of resolved congeners; 3) Extracted samples from the different time points in addition to Aroclor standards were run multiple times during separate sampling sequences in order to ensure reproducibility and accuracy over time.

In the data analysis, the mass of the individual congeners was measured on the GC in the 1 ml extracts. This congener mass was converted to mass percent by dividing the mass of the congener by the total mass of all detected congeners. This mass percent was then divided by the molecular weight of the specific congener in order to obtain the relative molar presence. The molar percent was subsequently obtained by dividing the relative presence of the specific congener by the sum of molar presence for all detected congeners. The molar percent for all congeners in one homolog group were added in order to get the molar percent for this homolog group and similar for the other homologs. The chlorines per biphenyl were obtained by calculating the relative number of chlorines in a homolog group by adding the sum of the measured masses of all congeners in a homolog group and multiplying by the number of chlorines and subsequently divide by the sum of all relative chlorine numbers. The data obtained from each of the three replicates were averaged and the standard deviation was calculated based on this.

All congeners were included in the analysis no matter how low the detected concentration was in the source data from the GC analysis. The detection level was defined as the lowest concentration of the individual PCB congeners used to establish the calibration curve used in the GC method, which was 4 µg per l. If a specific congener was detected at day 0, but not at day 200, the value for day 200 would be defined as 0 µg per l and this concentration would be used in the subsequent calculations.

4.7 DHPLC (Denaturing High Performance Liquid Chromatography)

The diversity of the PCB dechlorinating organisms in the mesocosm samples was examined by using the DHPLC based assay according to (Kjellerup, *et al.*, 2008). An initial run was used to identify individual PCR fragments and determine their retention times. Individual peaks were eluted for sequencing from a subsequent run and collected with a fraction collector and sequenced using the BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA). Sequences were analyzed using the automatic nucleic acid aligner in the ARB software package.

5. Results and Discussion

A. Establishment of anaerobic dechlorinating biofilm inoculum

A1. Cultivation of anaerobic bacterial inoculum using DF1

The bacterium *Dehalobium chlorocoercia* DF1 (DF1) was used as a model organism for anaerobic PCB dechlorination and several cultures were grown during the project both for observation of growth mode, methods development (imaging by FISH, SEM etc.) as well as preparing inoculum for the mesocosm experiment. In Figure 2 an example of data from growth of DF1 cultures is shown. On day 0 (immediately after transfer), the qPCR data showed that the cell numbers in the cultures ranged between $1.3x10^5$ and $7.3x10^5$ cells/ml (Figure 2). By day 35, all cell numbers had decreased, likely due to a lag phase where the bacteria adapt to the new conditions. Between day 35 and day 70 all cultures increased in cell numbers (except culture no.4) by an average of $3.6x10^5$ cell/ml. Overall, cultures 1, 2, 4, and 6 demonstrated net increases in numbers of dechlorinating bacteria averaging $2.12x10^5$ cells/ml. Cultures 3 (no GAC) and 5 (GAC) showed a net decrease in bacterial numbers of $5.45x10^5$ and $3.83x10^5$, respectively (Figure 2).

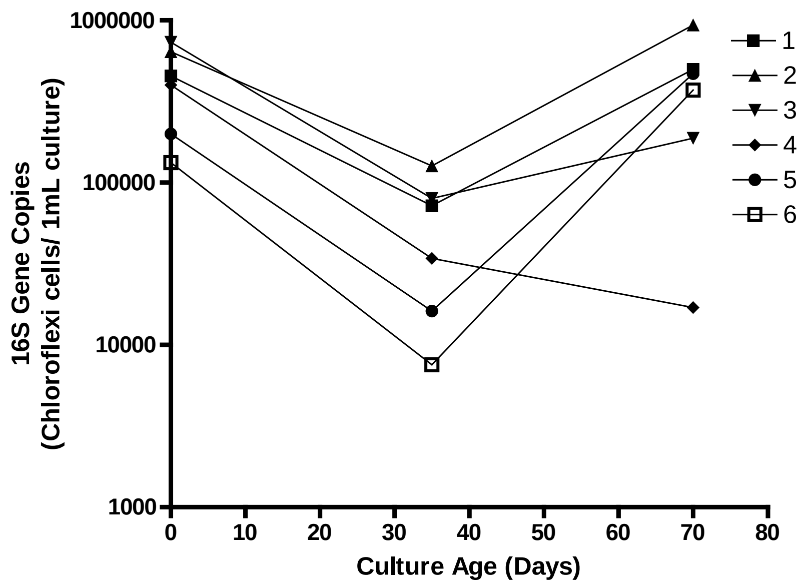

Figure 2. Numbers of anaerobically dechlorinating DF1 bacteria cultures used for sediment mesocosm inoculum. A total of six cultures were applied for this purpose. On Day 57 of the cultures GAC was added to cultures no. 4, 5 and 6.

To observe the dechlorination activity of the DF1 cultures the relative mol% dechlorination of PCB-61 to PCB-23 (para-dechlorination product of PCB-61) was calculated based on the amount of each congener obtained through extraction and analysis by gas chromatography. Cultures 1, 2 and 3 showed a decrease in mol% PCB61 with subsequent increases in %PCB 23 until day 56 with the fastest rates of turnover occurring between day 0 and day 35 (Figure 3: A1, A2). Between day 56 and 70, %PCB 61 increased in cultures 1 and 3 (Figure 3 - A1, A2), which likely was due to loss of PCB-23 during analysis, since the cultures were grown in a closed system. In the same time period the dechlorination seemed to continue in culture 2 seen as increased mol% of PCB-23 (Figure 3: A1, A2).

The cultures 4, 5 and 6 were treated the same was as cultures 1, 2 and 3 until day 56, when activated carbon was added at 3% to each of the cultures. At day 0, the results showed that the cultures 5 and 6 contained approximately 35% and 25% PCB-23, respectively, whereas culture 4 contained approx. 10% PCB 23 (Figure 3). Before day 57, culture 4 demonstrated decreases in %PCB-61 with the fastest rate of turnover occurring between day 35 and 56. Cultures 5 and 6 showed limited activity until day 35, when both cultures showed dechlorination with increases in %PCB-23 until day 56 (Figure 3: B1, B2). Cultures 4-6 all seem to display the highest dechlorination rates of PCB 61 to PCB 23 between days 30 and 56. Unexpectedly, all cultures in which GAC was added (4-6) presented marked increased % PCB 61 and decreased % PCB 23 after day 57 (Figure 3: B2, B2). However, the reason for this is very likely that equilibrium between the added activated carbon and the PCBs had not been reached after only two weeks following the addition and not that the cultures became inactive. This is supported by the increased cell numbers (by qPCR, Figure 2) and by the imaging analysis showing that the bacteria had formed biofilm that were present at the surface and were active.

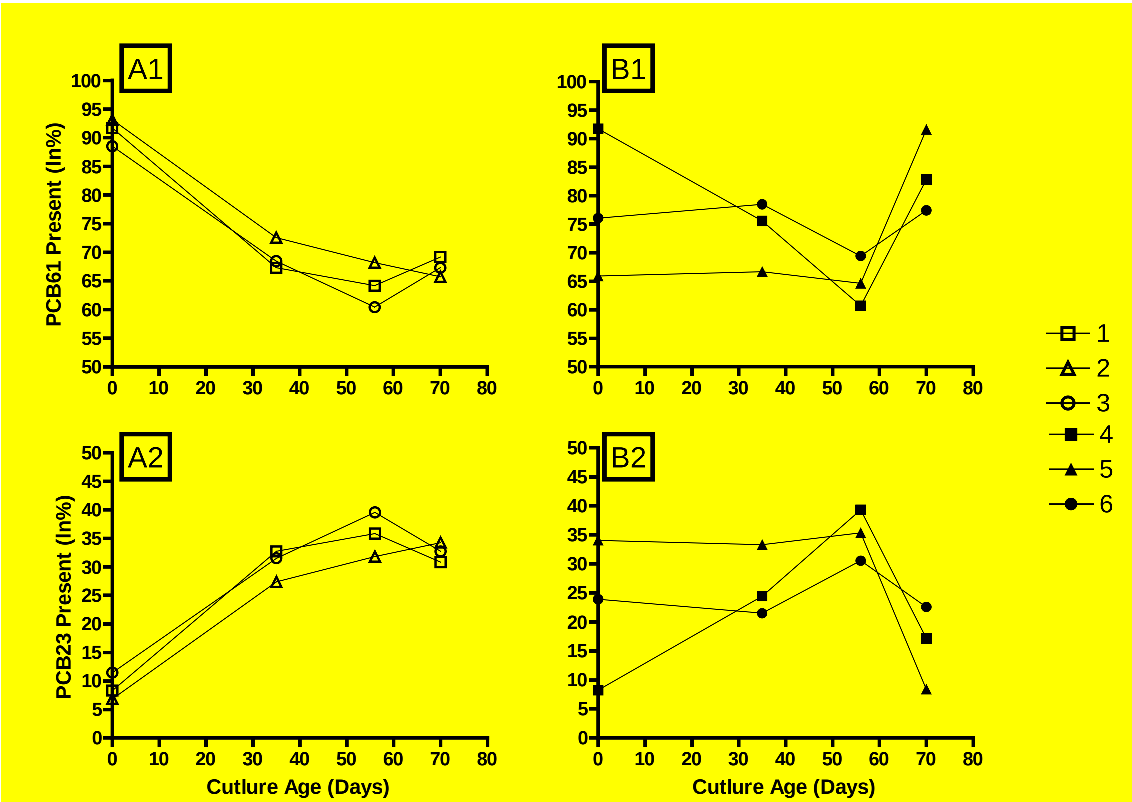

Figure 3. Comparison of the growth of DF1 cultures in the absence (A1, A2) and presence (B1, B2) of activated carbon (same cultures as in figure 2). All cultures were transferred at Day 0 and grown in minimal media with 50 ppm PCB-61 (2,3,4,5-PCB). PCB-23 (2,3,5-PCB) is the product of double-flanked para-dechlorination of PCB-61 by DF1. Activated carbon was added to cultures 4, 5 and 6 on day 56.

Before the addition to the sediment mesocosms, DF1 cultures 1-6 were sampled for imaging on day 70 by scanning electron microscopy. Images confirmed that biofilm had formed on the GAC particles to a greater extent (estimated, not measured with image analysis) than in the cultures without GAC (Figure 4). Clumps of DF1 had formed and could be characterized as biofilms in cultures 1-3, but these clumps were not as big and many as the GAC biofilms, cultures 4-6 (Figure 4). Biofilm clumps form on GAC did not discriminate between seemingly smooth or rough surfaces.

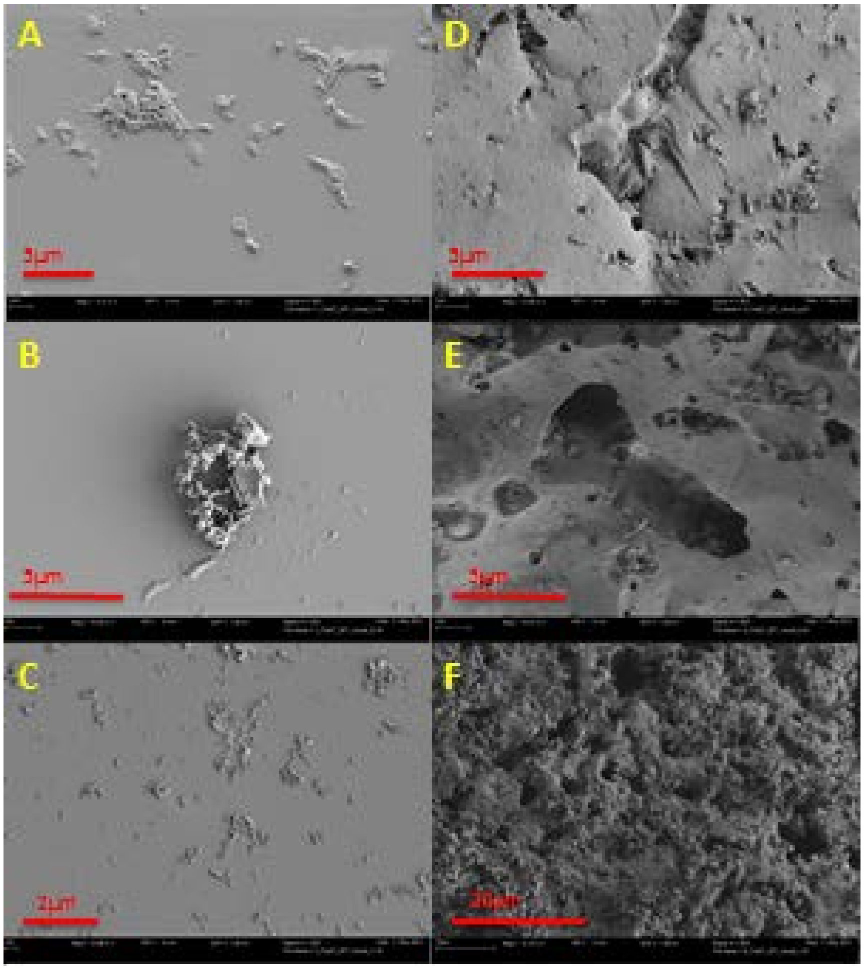

Figure 4. SEM of anaerobic DF1 cultures 1-3 without Activated carbon (left: A, B, C) and 4-6 with GAC (right: D, E, F) at day 70. GAC was added to cultures 4-6 two weeks prior to imaging. Drops of cultures were dried onto silicon wafers and prepared according to SEM protocol (ICAL, MSU Bozeman, MT).

<u>A2. Analysis of the biofilm composition with respect to elements</u>

In addition to obtaining high resolution images with SEM, it is also possible to examine the 5 µm top layer of the biofilm sample being analyzed for elemental composition. In this project we utilized this feature to analyze the top layer of the activated carbon particles and biofilm, respectively to locate the presence of potentially adsorbed PCBs by looking at the chlorine presence (Figure 5, Table 2). The results showed that the chlorines (showing the presence of the PCBs) was present in the biofilm layer, when a biofilm was present, whereas the activated carbon adsorbed the PCBs as expected, when biofilm growth was absent. The close proximity between the dechlorinating cells in the biofilm and the PCBs that was one of the expectations for developing the delivery system was thereby verified.

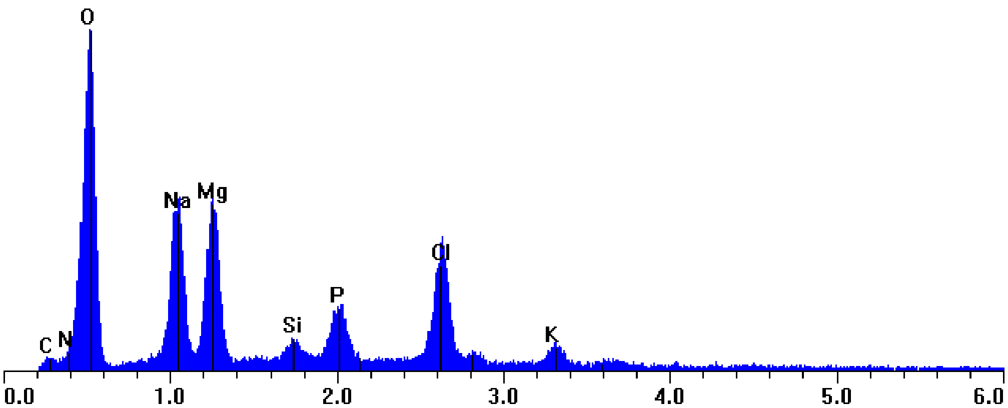

Figure 5. Elemental analysis of a biofilm of the anaerobic dechlorinating DF1 biofilm from Figure 6. The presence of the chlorine peak shows the presence of PCBs in the biofilm.

Table 2. Elemental analysis of the anaerobic DF1 biofilm shown in Figure 4. Note that the chlorine content is 6.7%, whereas this content is zero for a clean surface.

Element	C	N	O	Na	Mg	Si	P	Cl	K
Weight%	1.81	0.71	60.88	15.78	10.19	0.71	2.05	**6.68**	1.18

<u>A3. Success criterion 1:</u>
To establish inoculum consisting of anaerobically dechlorinating biofilms that can be used as a microbial inoculum delivery system in sediment. Two methods were examined: biofilm covered activated carbon particles (documented here) and enriched wastewater sludge biofilm (documented below).

In has in this section been shown that it is possible to establish anaerobic biofilm that attach and cover activated carbon particles, while the bacteria are actively dechlorinating PCBs. The methods and protocols for performing this culture work have been established and the obtained cultures can subsequent be applied as microbial inoculum and be delivered as a 2-in-1 system to the sediment for bioaugmentation purposes. The methods applied to verify the biofilm growth will be discussed in section A2 below. This criterion for successful completion was therefore fulfilled.

B. Development of tools that can be applied to evaluate biofilm formation

<u>B1. Analytical methods for analysis of biofilms</u>
As described in the Materials and Methods section, analytical methods for DNA extraction and subsequent PCR based analysis as well as PCB extraction and analysis have already been performed in previous studies, which is why methods development was not needed in this project.

B2. Development of method for SEM analysis of biofilm

In February 2012 the PI visited the imaging facility ICAL at Montana State University (subcontractor on this project) in order to develop the method for imaging with scanning electron microscopy (SEM) the bacterial biofilms on the activated carbon surfaces as well as the aggregates in the liquid medium. We analyzed numerous samples containing anaerobic cultures of DF1 in order to visualize the activated carbon with and without bacterial biofilm and also to perform elemental analysis of the biofilms/surfaces that were observed. The elemental analysis can be performed on the outer layer (5 µm) of the biofilm/surface coating and enable the observation of chlorine atoms attached to the surface in the form of polychlorinated biphenyl molecules.

The images clearly showed that biofilms form on the activated carbon surfaces and that they are embedded in matrix material that is not made up by bacterial cells. This matrix can consist of polysaccharides, proteins and other organic substances as well as PCBs in this case. The origin of the carbon atoms cannot be distinguished with this analysis, but the detection of chlorine atoms show that the PCBs are present in the examined biofilm layer. The analysis cannot distinguish between specific PCB congeners, but chlorines present as the result of dechlorination would not be present in the biofilm, since they are soluble molecules and therefore would be present in the bulk water. The images (Figures 6-8) are representative for the different conditions that were tested in biofilm formed on GAC surfaces (Figure 6), aggregates of bacteria formed without GAC (Figure 7) and activated carbon without bacteria present (Figure 8).

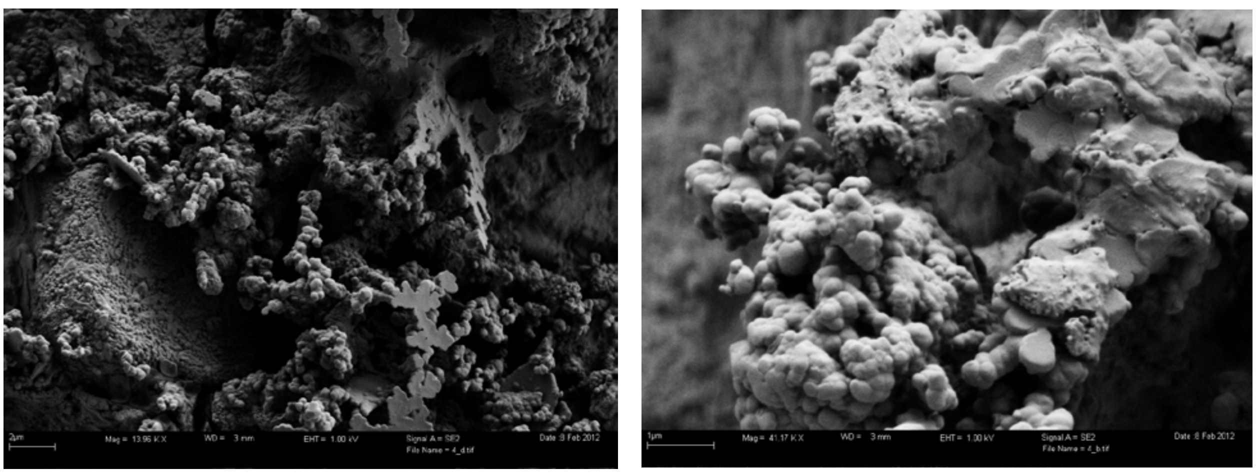

Figure 6. Images from SEM with activated carbon showing the dechlorinating bacteria embedded in the biofilm matrix on the surfaces of the activated carbon (Note: The figures show different magnifications).

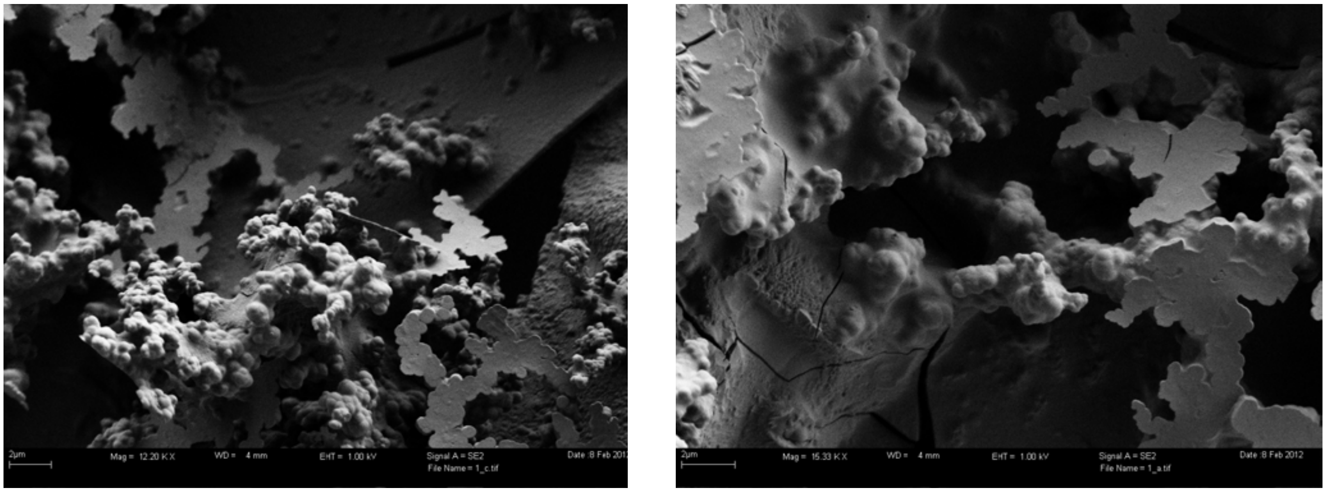

Figure 7. SEM showing aggregates of DF1 forming without activated carbon (Note: The figures show different magnifications).

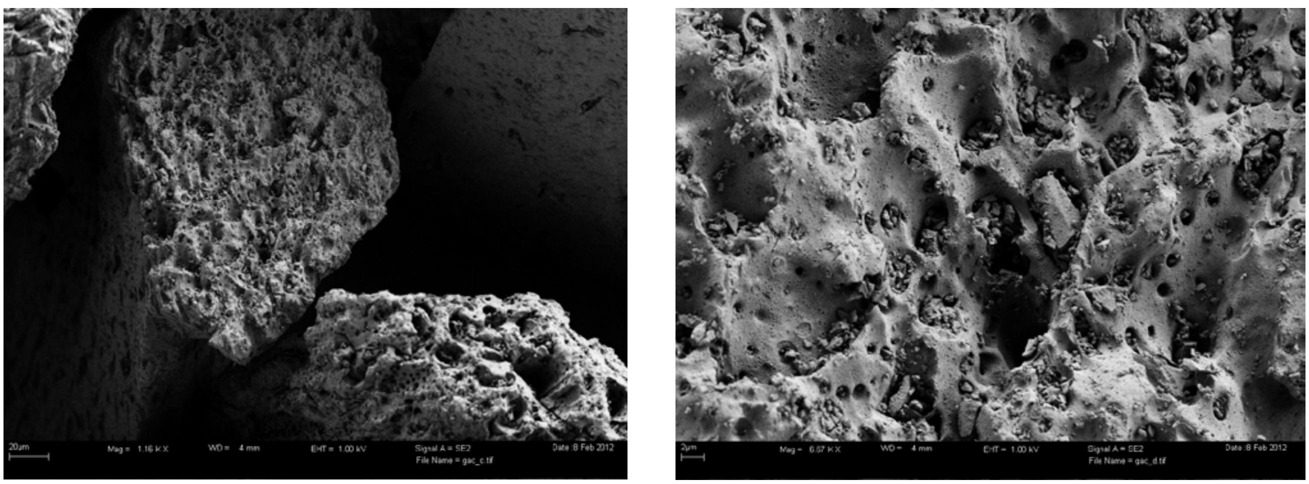

Figure 8. SEM images of the activated carbon surfaces as they look without being exposed to bacteria or culture media (Note: The figures show different magnifications).

<u>B3. Development of fluorescent methods for analysis of biofilm</u>
In this project a number of fluorescently based methods were developed and applied:

- Staining of all bacteria using the DNA specific stain DAPI.
- Specific staining of active bacteria by fluorescence in situ hybridization using traditional as well as the new PNA-FISH method.
- In situ analysis of active biofilms in the intact and undisturbed wet sample using the newly developed technique applying the specific DNA stain SybrGreen together with a wet mount objective on a confocal laser scanning microscopy (CLSM).

The application of all the above methods was successful and the images in the Figures 9-11 document this.

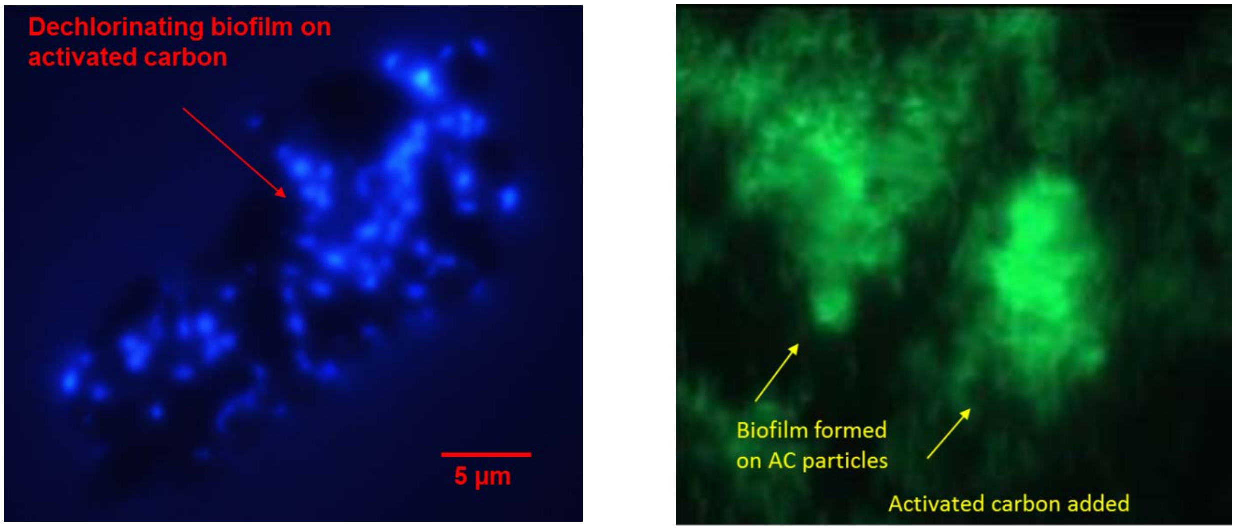

Figure 9. Left: DAPI staining; Right: PNA-FISH of activated carbon particles covered with anaerobic DF1 biofilm.

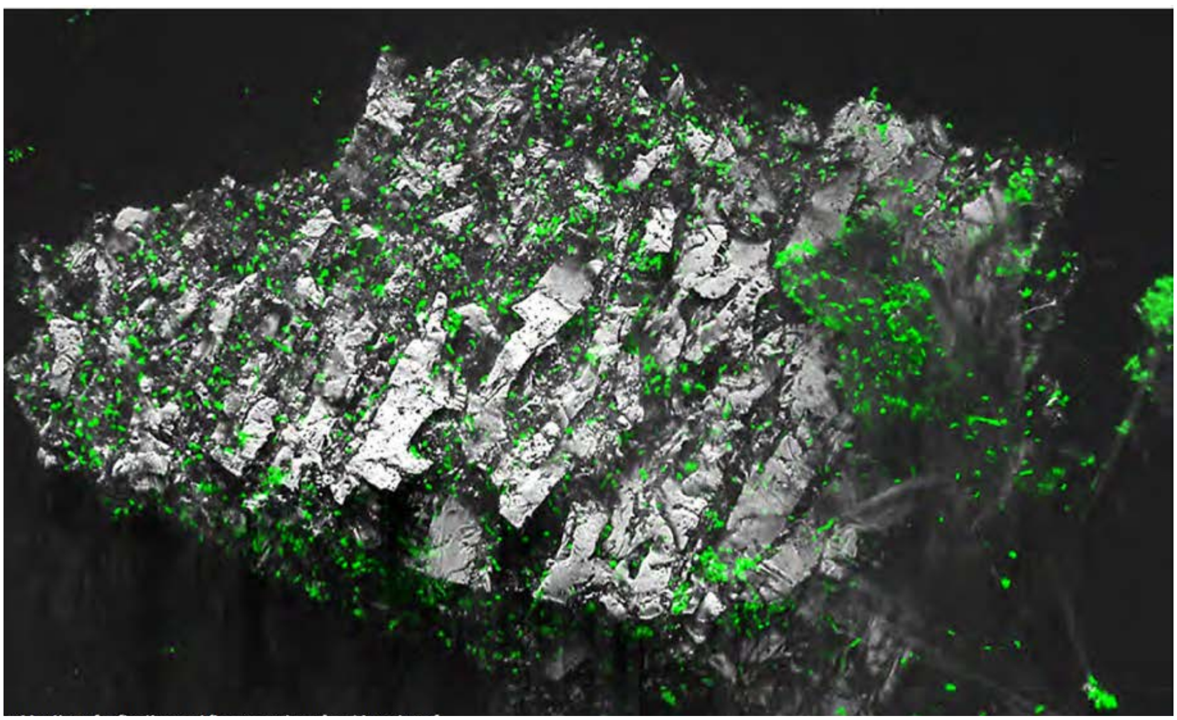

Figure 10. The image shows DF1 biofilm formed on the surface of an activated carbon particle. The bacteria were labeled with the DNA specific stain SybrGreen that only targets DNA (i.e. bacterial cell material) and not the background such as activated carbon and/or media components. This method was not included in the original proposal, since it was developed in the meantime, but showed to be very valuable.

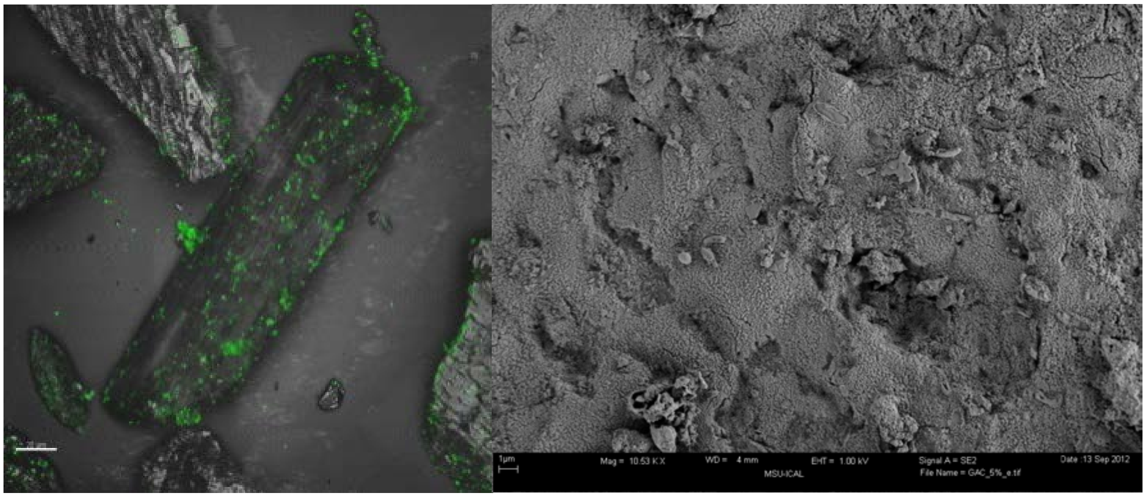

Figure 11. Comparative image analysis of DF1 Biofilm. Left: Sybr Green confocal image (Betty Pitts, CBE, MSU). Right: SEM of the same sample of DF1 biofilm (ICAL, MSU).

This new SybrGreen-CLSM imaging technique (Figures 10 and 11) benefits from the fact that only bacteria are targeted and thus provides more accurate information than SEM in terms of how and where DF1 forms biofilms on the activated carbon particles. Compared to SEM precipitates from media as well as background from sediment samples provides little to no interference, whereas it can be difficult to distinguish bacteria from the background at SEM images of the same samples. In Figure 11 this is illustrated by applying the SybrGreen CLSM technique in parallel with SEM on the same sample. The image demonstrates the interference of media precipitate in providing accurate and detailed DF1-biofilm imaging. Besides the difficulty in clearly imaging the bacteria, the SEM technique is inherently more difficult than SYBER Green CLSM to master, because it requires high magnification that only small fields of view of the activated carbon particle can be visualized, whereas the aforementioned SybrGreen-CLSM technique images the entire particle while bacteria are still visible.

<u>B4. Success criterion 2</u>
To develop tools that can be used to analyze biofilms formed on a surface with regards to bacterial numbers and populations as well as PCB dechlorination activity.

Based on the results obtained in this current project and documented in this section together with already developed techniques by the PI and others, a “tool box” for analyzing biofilms forming on activated carbon particles as well as sludge biofilms is now existing that can be applied in potential bioremediation projects with anaerobic dechlorinating biofilms. This criterion for successful completion was therefore fulfilled.

C. Mesocosm study: Application of biofilm covered activated carbon particles and enriched sludge biofilms as delivery systems

As shown previously, biofilm covered activated carbon particles were formed and added to anaerobic mesocosms with sediment from Grasse River, NY. In addition, the effect of enriched cultures from wastewater sludge was also tested. During the project period results showed a remarkable abundance of dechlorinating bacteria in this wastewater system in addition to the structure of sludge being very similar to the delivery vehicle based on activated carbon proposed in this project (Figure 1 and 19). Grasse River sediment has traditionally been contaminated with Aroclor 1248 (A1248) due to the industrial activities taking place at this site (Kjellerup, *et al.*, 2008). In this experiment the sediment was mixed to obtain a final concentration of 50 ppm in order to test the delivery systems under conditions, where dechlorination likely would happen faster compared to a non-spiked situation with low environmental concentrations. This was desirable due to the relatively short project period. The dechlorination was monitored over 200 days (still ongoing) and the results can be seen in Figures 12-20 for both the biofilm covered activated carbon particles as well as the enriched sludge system.

C1. Dechlorination activity in sediment mesocosms

In figure 12, the overall decrease in chlorines per biphenyl from day 0 to Day 200 is shown. By using the measure “chlorines per biphenyl” any dechlorination process that would occur in the sediment will be reported as actual chlorine loss independent of the affected homolog or congener. The loss in chlorines per biphenyl over the 200 day period was calculated by subtracting the number of chlorines for D0 from D200. The number of chlorines for each of the time points was calculated as follows: The number of chlorines in a homolog group was determined by summing up all the measured congener masses in the group and multiplying by the number of chlorines present for the homolog. This number was then divided by the sum of all the chlorine numbers to get the overall number of chlorines per biphenyl for this sampling point. The data obtained from each of the three replicates for the time point were averaged and the standard deviation was calculated based on this. The results show that dechlorination occurred in all mesocosm except for the negative control with autoclaved sediment. The highest decrease in overall dechlorination was observed in the mesocosms, where anaerobic biofilms had been applied for bioaugmentation either in the form of biofilm covered activated carbon particles or the enriched sludge biofilm. There was limited activity taking place in the mesocosms, where the liquid culture of the anaerobic dechlorinating organism DF1 or sterile filtered spent media from DF1 had been added. Also, addition of autoclaved sludge did not enhance the dechlorination activity showing that the nutrients that were supplied together with the enriched biofilm did not increase the dechlorination in the sediment.

When the biofilm inoculum based on the wastewater sludge was applied (Figure 15) the dechlorination of more extensively chlorinated congeners was more pronounced than when DF1 biofilm was used as a delivery vehicle. The diverse biofilm population was able to dechlorinate an even wider range of congeners in the sediment (bars below x-axis) and formed predominantly mono- and di- chlorinated products. This delivery system showed the highest dechlorination rate compared to the other mesocosms. In addition, it was shown that this high rate was based on dechlorination of a wide range of congeners. In the environment this would mean that extensively chlorinated congeners would be targets for dechlorination independent of the configuration due to the presence of the mixed biofilm population in the enriched sludge biofilm. The mixed biofilm population was not limited to a specific dechlorination patterns as was the case with the DF1 biofilm, where predominantly double flanked *meta* and *para* dechlorination can take place (May, *et al.*, 2008). Instead the mixed biofilm delivery system from enriched sludge resulted in the formation of mono- and di-chlorinated congeners (2, 4, 22'/26, 23', 23/24', 22'6 -PCB) that can be attacked by aerobic PCB degraders and eventually mineralized.

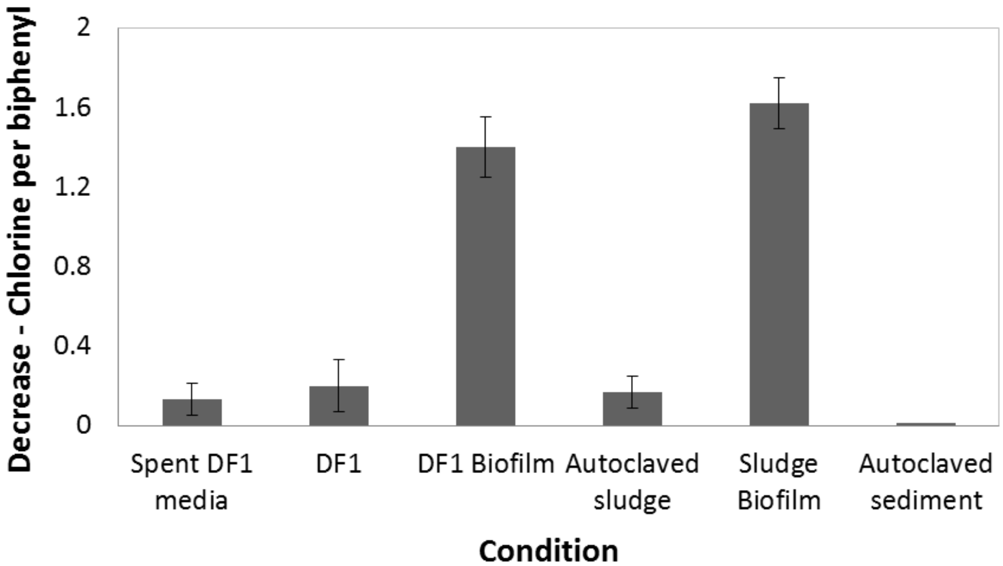

Figure 12. The decrease in chlorines per biphenyl in the mesocosm experiment involving anaerobic conditions with DF1 biofilm as well as enriched sludge biofilm.

These results are also evident, when the dechlorination rate was calculated for the 200 day long period that the mesocosms so far have been evaluated (Figure 13). The dechlorination rate for A1248 in biofilm augmented mesocosms were 7.0 and $8.1 \cdot 10^{-3}$ chlorines per biphenyl per day, respectively for biofilm covered activated carbon particles and enriched sludge biofilm compared to less than $2 \cdot 10^{-3}$ chlorines per biphenyl per day for undisturbed sediment.

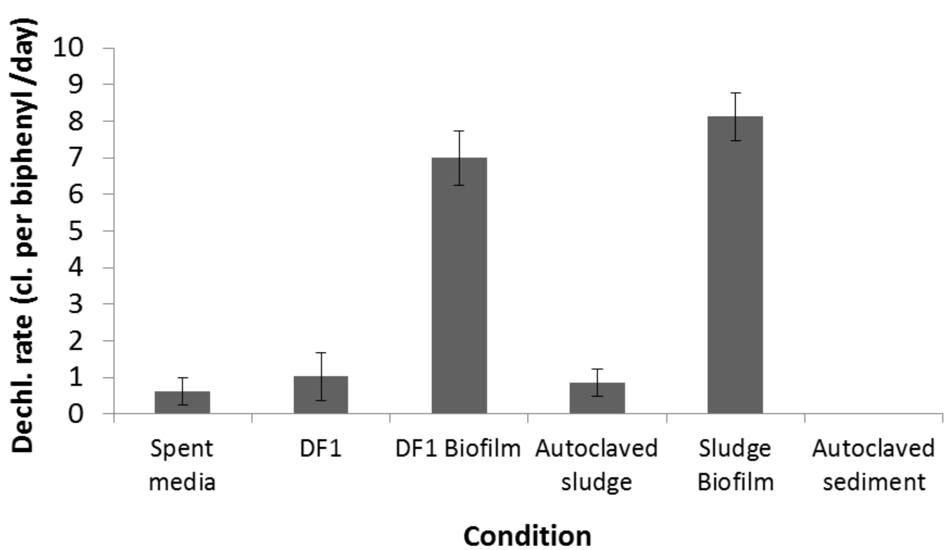

Figure 13. The dechlorination rates for anaerobic activity in the mesocosm experiment involving anaerobic conditions with DF1 biofilm as well as enriched activated sludge biofilm.

A detailed look at the dechlorination taking place in the sediment that was augmented with the anaerobic DF1 biofilm can be seen in Figure 14. Here, the change in mol% for the individual congeners is shown over the 200 day period. This analysis was performed to get a detailed look at which individual congeners contributed to the overall loss of chlorines (reported in Figure 12) after the introduction of the dechlorinating microorganisms in the inoculum. In this way it was possible to determine whether the overall chlorine loss was based on a wide variety of congeners or only a few. If the chlorine loss was a result of dechlorination of a wide variety of congeners, this would indicate that the process would be robust and not be affected by potential limiting factors. On the other hand, if the chlorine loss was a result of dechlorination of a few highly chlorinated congeners, the dechlorination process would be sensitive and would likely decrease over time and finally stall, since the source congeners for the chlorine loss would be depleted.

Figure 14 shows the removal of more extensively chlorinated congeners (below the x-axis) and the dechlorination products (above the x-axis). The inoculation with this biofilm delivery vehicle showed that mono-, di- and tri-chlorinated congeners were formed as a result of dechlorination from a wide range of extensively chlorinated congeners. This shows that DF1 did not prefer specific congeners and also likely promoted enhanced dechlorination activity by the indigenous dechlorinating population due to formation of less extensively chlorinated byproducts. The accumulation of mono-, di- and tri-chlorinated congeners shows that aerobic degradation did not take place in this mesocosm, since this group of congeners likely would have been degraded by aerobic PCB degraders (Bedard, *et al.*, 1986). Instead, the potential for aerobic degradation would be present if the environmental conditions changed to aerobic in zones of the sediment since the PCB degraders are ubiquitous in the environment. This would provide complete removal of the PCBs.

The evaluation of the loss of chlorines in the anaerobic mesocosms was based on the relative presence of the individual congeners in comparison to the total concentration of the PCB congeners. In order to close the mass balance before and after the introduction of anaerobically dechlorinating inoculum, the GC analysis and subsequent data analysis should be based on absolute amounts. This would be even more important if aerobic processes were introduced, since chlorines could disappear from the system due to break down of the biphenyl ring. In a dynamic system with both anaerobic and aerobic processes taking place, the mass balance would therefore require inclusion of aerobic degradation products such as chlorobenzoic acids in addition to anaerobic dechlorination products. Analysis of chlorobenzoic acids could be performed using high-performance liquid chromatographic (HPLC).

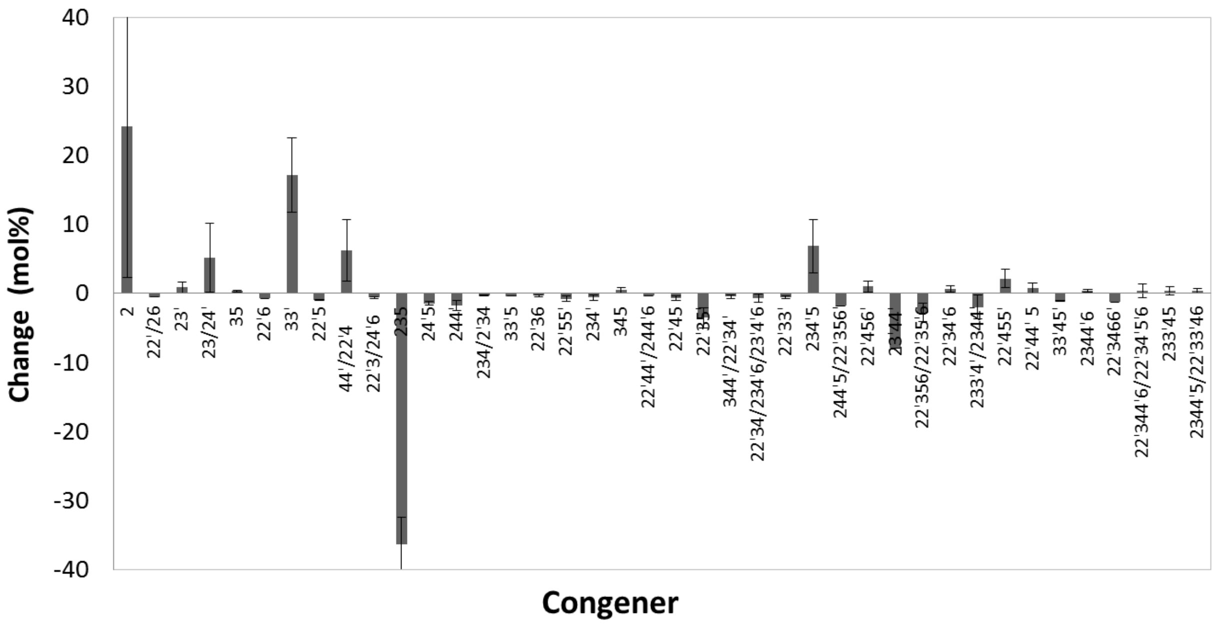

Figure 14. Change in individual congeners in the mesocosm inoculated with anaerobic biofilm covered activated carbon particles. Congeners with a change >0.25 mol% were included.

In order to evaluate whether the differences in mol% over the 200 day period were statistically significantly different, a two-way T-test was performed. On Figure 14, 40 congeners where shown with a difference in mol% above 0.25%. Based on the T-test, 9 of the 40 congeners (23%) showed a p-value below 0.3 and 38 of 40 (95%) congeners showed p-values below 0.45 (Figure 15: 9% vs. 95%). When all the detected congeners were included in this statistical analysis, the results showed that 17% of the congeners had p-values below 0.3 and 94% had p-values below 0.45 (Figure 15: 18% vs 94%). The statistical evaluation shows that between 9 and 23% of the congeners showed a difference over the 200 day period due to dechlorination that was within the acceptable 30% variance that has been defined by the EPA. If this level of variance was increased to 45% approximately 95% of all the individual congeners would fall within the acceptable variance level. Overall, the p-values were lower for the largest mol% differences in Figure 14 and 15 indicating that the changes that were observed were real changes and not an artifact due to instrument variability or other parameters influencing the results during analysis. When the statistical significance was tested for the chlorines per biphenyl change for the anaerobic biofilm (data for Figure 14) and the anaerobic enrichment from the sludge biofilm (Figure 15), the results showed a p-value for the anaerobic biofilm of 0.19 and 0.006 for the sludge biofilm, respectively. Both of these values are below the 30% variance level accepted by the EPA. Altogether the results show that parameter chlorines per biphenyl showed acceptable results with regards to EPA standards, whereas the individual congener analysis showed that 9-23% of the congeners were within the 30% level.

The anaerobic biofilm inoculum in both cases caused enhanced dechlorination of A1248 in the sediment mesocosms, but the mixed population originating from the enriched sludge was able to dechlorinate a wider range of large congeners and produce fewer congeners as dechlorination products. This was due to the diversity of dechlorinating bacteria in the sludge biofilm, where six to seven major dechlorinating phylotypes were detected (Figure 17). The results also showed that the high dechlorination rate in the sediment was caused by a more diverse and active biofilm inoculum, since the number of dechlorinating bacteria was approximately the same in all mesocosms throughout the experiment (Figure 16).

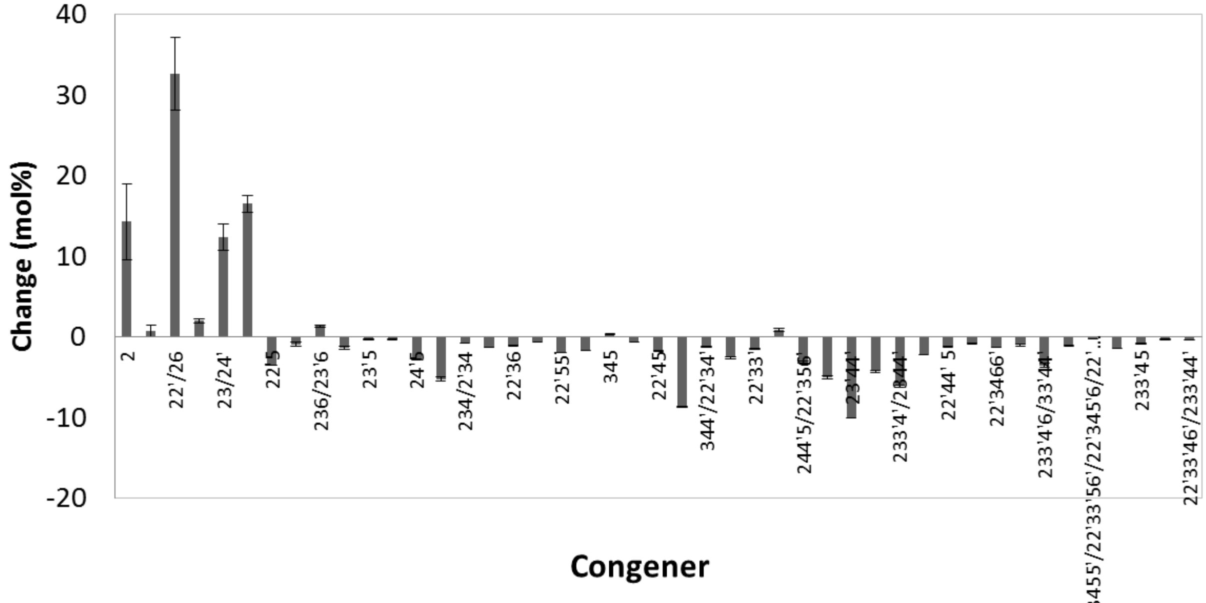

Figure 15. Change in individual congeners in the mesocosm inoculated with anaerobic biofilm enriched from wastewater sludge. Congeners with a change >0.25 mol% were included.

Adsorption of PCBs to the activated carbon particles was evaluated in the control mesocosms that were included for abiotic processes (autoclaved sediment, no inoculum) and for background dechlorination in the presence of Aroclor 1248 (no inoculum). The results from these mesocosms based on the parameters mol% and chlorines per biphenyl showed that recovery of congeners reflected the original composition of Aroclor 1248. This means that preferential adsorption of for instance highly chlorinated congeners was not observed and that irreversible adsorption of specific PCB congeners did not occur. However, since the relative presence of congeners was evaluated in the analysis (in % of the total amount of Aroclor 1248) instead of absolute amounts, it was not evaluated whether irreversible adsorption of PCBs on a mass basis occurred ("irreversible" within the time period of the experiment). This could be evaluated in potential future studies, where more advanced analytical methods for PCB extraction and analysis could be applied.

<u>C2. Abundance and diversity of dechlorinating biofilm populations</u>

The total number of dechlorinating bacteria was evaluated in the sediment mesocosm to examine if the inoculum would remain in the sediment throughout the experiment. In the beginning of the experiment, approximately $3 \cdot 10^5$ cells per g sediment was present in all mesocosms (Figure 16). This increased for all mesocosms to approximately $1 \cdot 10^7$ cells per g sediment at day 150, which was the maximum observed. After this time the numbers decreased to approximately $2 \cdot 10^6$ cells per g sediment at day 200. The lag phase in the beginning of the experiment was likely due to the mixing of the mesocosm sediment with cultures, A1248 and other additions that might have introduced a change in conditions such as oxygen in some pockets or areas of oxygenated liquid. These unfavorable conditions disappeared over time due to overall anaerobic microbial activity (respiration) removing the available oxygen in the sediment thus creating complete anaerobic conditions for the dechlorinating populations.

The inoculum added as DF1 biofilm was shown to sustain the environmental conditions and multiply approximately 100 times after the initial lag phase. When the enriched activated sludge biofilm inoculum was added to the sediment mesocosm approximately $1.9 \cdot 10^5$ cells per g sediment were observed at day 0. This number doubled over the course of the experiment and was at day 200 at $2.8 \cdot 10^6$ ($\pm$ $5.6 \cdot 10^5$) cell per g sediment. This anaerobic biofilm inoculum was also able to survive and establish an active dechlorinating population when added to the sediment mesocosm.

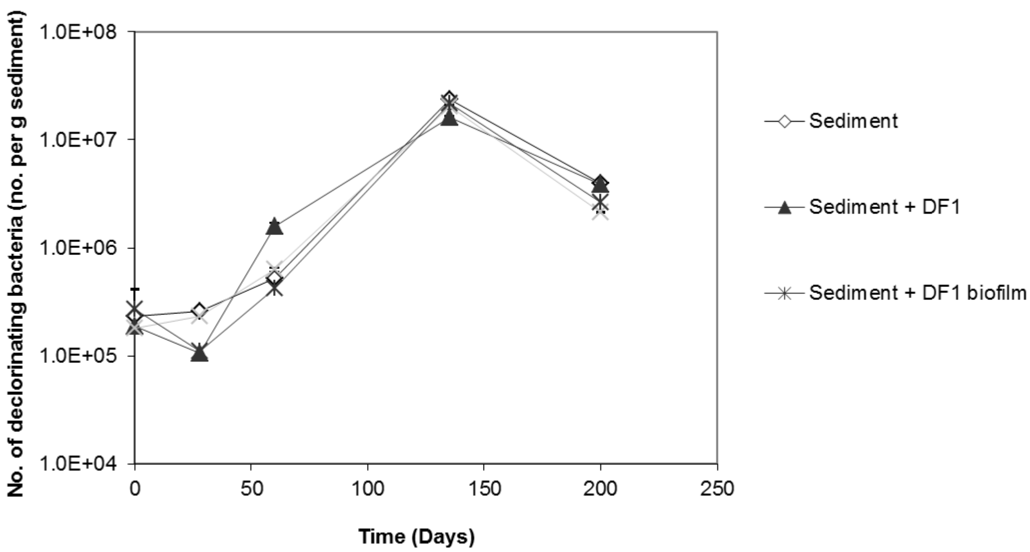

Figure 16. The number of dechlorinating bacteria in mesocosm samples inoculated with anaerobic cultures of DF1 and enriched sludge biofilms together with the relevant controls over the course of the experiment.

The diversity of dechlorinating bacteria was evaluated via DHPLC in the sludge biofilm inoculum and showed the presence of six to seven dominant phylotypes, which can be seen as individual peaks at the chromatogram (Figure 17). The single congener PCB-116 (2,3,4,5,6-PCB) as well as the commercial Aroclor mixtures A1242 and A1248 were applied for enrichment experiments and it can be seen that the diversity in the enriched sludge biofilm inoculum did not depend on the type of PCB that was used for enrichment. The results also show that the diversity in the two types of sludge (activated sludge vs. dewatered sludge) that was examined did not vary significantly.

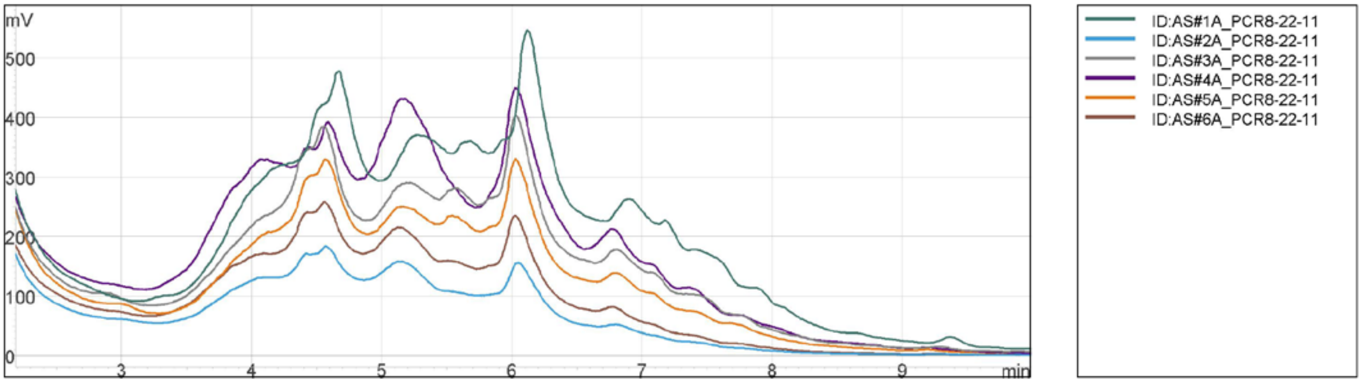

Figure 17. The diversity of dechlorinating bacteria in activated sludge (1, 3, 5) and dewatered sludge before it enters an anaerobic digester (2, 4, 6) exposed to 50 ppm of PCB-116 (1,2); A1242 (3, 4); A1248 (5, 6) and analyzed via DHPLC. The peaks indicate the presence of distinct phylotypes of dechlorinating organisms and six to seven dominant phylotypes were present.

An investigation of the abundance of dechlorinating bacteria in the wastewater plant showed surprisingly high numbers (Figure 18). The two types of sludge that were examined with the DHPLC (Figure 17) show the highest abundance of dechlorinating bacteria at this wastewater treatment plant (normalized to gram of dry biomass). However, even in the incoming wastewater the dechlorinating bacteria were present even though the concentration of PCBs at all times was below the detection limit of 0.5 ppb (Phillips, 2012). The abundance of dechlorinating bacteria was significantly reduced, when the sludge passed through anaerobic digestion as the final process, but there was still approximately $1 \cdot 10^8$ cells per g dry sludge present, which is 10-100 times more than can be found in PCB contaminated sediment (Kjellerup, *et al.*, 2008). Other studies have also observed the capacity of dechlorination in biofilms that have not previously been exposed to PCB contamination (Macedo, *et al.*, 2007). These results showed that there was a potential for application of enriched anaerobic sludge biofilms as a delivery system for inoculum to sediment systems.

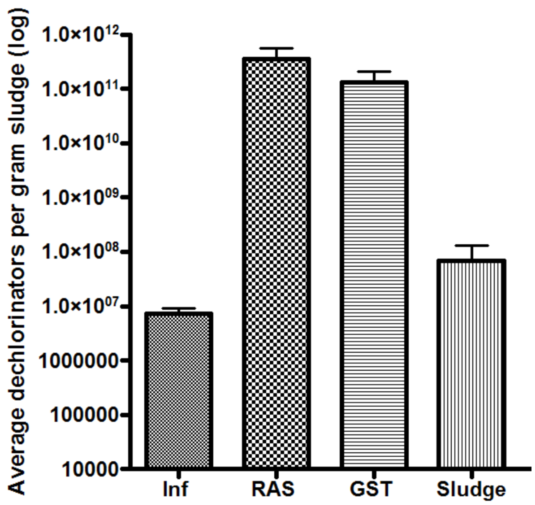

Figure 18. Number of dechlorinating bacteria present in wastewater and activated sludge at different sampling locations at Back River Wastewater Treatment Plant in Baltimore, MD. The numbers have been normalized to the dry matter content, since this varies significantly throughout the plant. Inf = Influent; RAS = Recirculating Activated Sludge; GST = Gravity Sludge Thickener; Sludge = Sludge after anaerobic digestion at thermophilic conditions (60-70C).

<u>C3. Microscopic evaluation of dechlorinating biofilm populations</u>

The biofilm delivery systems that were examined as inoculum in the sediment mesocosms were based on biofilm covered activated carbon particles (Figures 9-11) and enriched sludge biofilm (Figure 19). A combination of the DAPI and FISH techniques was applied to examine the presence of dechlorinating bacteria in the sludge biofilm. All bacteria (living and dead, blue), all living bacteria (green) and *Dehalococcoides* (a group of all dechlorinating bacteria, red) were stained. In the images (Figure 19), it can be seen that dechlorinating bacteria were present and as expected only made up a small part of the overall bacterial population. In other studies of PCB dechlorinating bacteria in sludge similar results have been obtained (Macedo, *et al.*, 2005). In addition to bacteria, biofilms are also made up of an exopolymeric matrix consisting of carbohydrates, proteins, lipids and extraneous organic and inorganic compounds adsorbed to the matrix. This surrounding material can be seen as “cloudy” and brighter stained material in Figure 19 compared to the very bright “dots” that are stained bacteria. The matrix is embedding the bacteria and can adsorb the PCBs together with the organic backbone of the sludge biofilm. However, this matrix is also ensuring an increased protection of the bacteria from the stresses that might occur as a result of the fluvial system that the biofilms are exposed to.

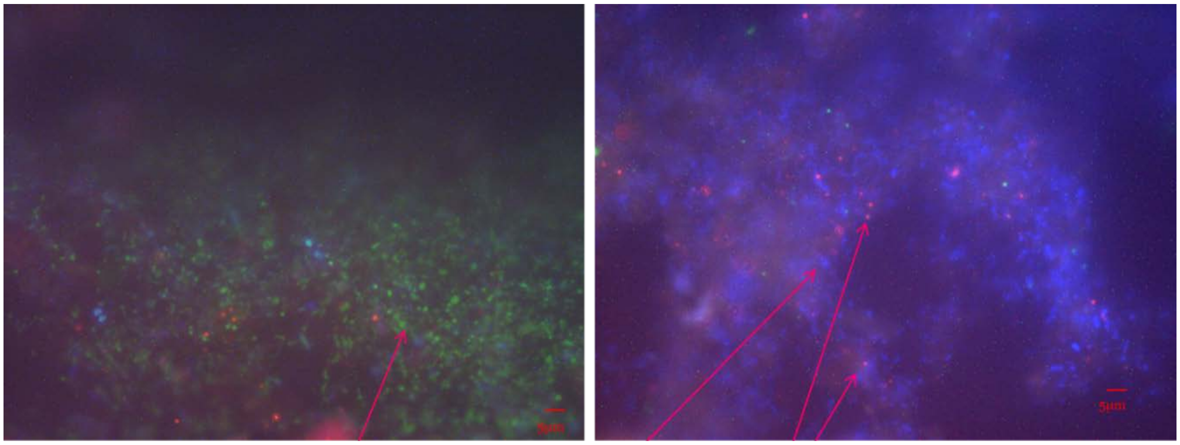

Figure 19. The presence of Dehalococcoides in activated sludge via FISH staining with a specific probe. Dehalococcoides only make up part of the total dechlorinating population, so the image is underestimating the complete number of dechlorinating bacteria in the sludge sample. Green = all living bacteria; Blue = All bacteria (living and dead); Red = Dehalococcoides.

When sediment samples from the mesocosms were analyzed for the presence of the augmented biofilm, these particles could be observed in the mixed sediment samples (Figure 20) thus supporting the qPCR results that showed that the biofilm inoculum remained in the sediment throughout the experiment. This also supported the fact that biofilm inoculum is a robust delivery system for bioaugmentation.

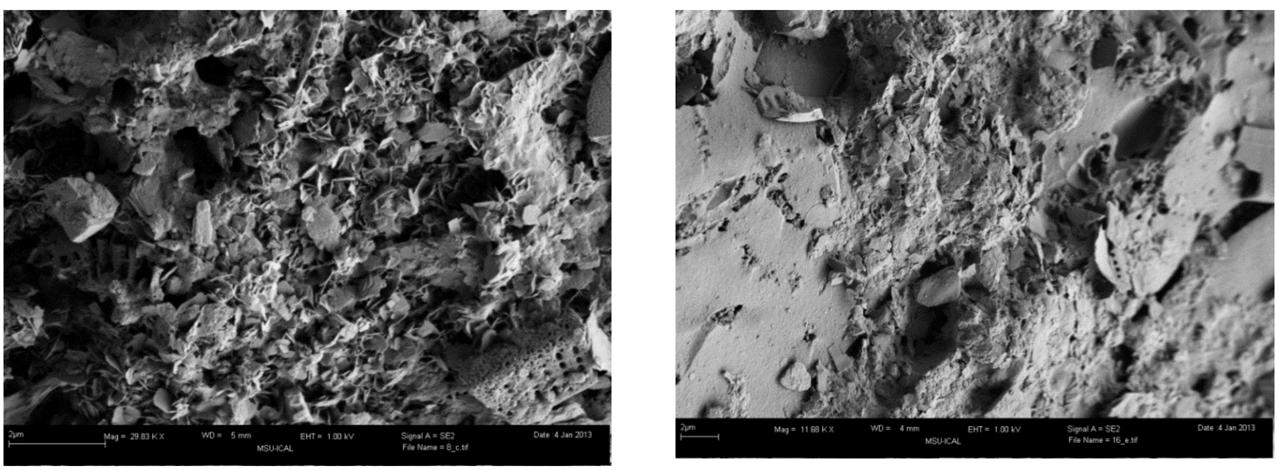

Figure 20. SEM images showing the presence of bacteria forming biofilms on surfaces of activated carbon particles in the sediment mesocosm. Left: The entire surface is covered with a biofilm, so the activated surface cannot be seen. Right: Biofilm is covering most of the surface but the activated carbon surface can be seen to the left in the image.

C4. Biofilm Formation by *Burkholderia xenovorans* strain LB400

The results reported in this section were based on experiments that were not included in the initial proposal that focused on the anaerobic dechlorination processes and should therefore be seen as preliminary data.

The aerobic PCB degrading bacterium *Burkholderia xenovorans* strain LB400 (LB400) was examined for its capability to form biofilms on activated carbon surfaces. It was grown in minimal media and 10 mM biphenyl was applied as substrate to cultures with and without 3% activated carbon. The growth was followed over several weeks by counting the total number of bacteria by DAPI. These results will at a later point be compared to qPCR results.

The initial results show that LB400 is an excellent and very fast biofilm former (Figures 21 and 22). Within only two weeks a biofilm had formed on the activated carbon particles and there were significantly more cells attached to the surface of activated carbon particles than were present in the liquid culture media. SEM images were obtained every 1-2 weeks in order to follow the biofilm formation on the activated carbon surfaces and it was very clear that the bacterial cells adhered very well to the surface and formed strong biofilms.

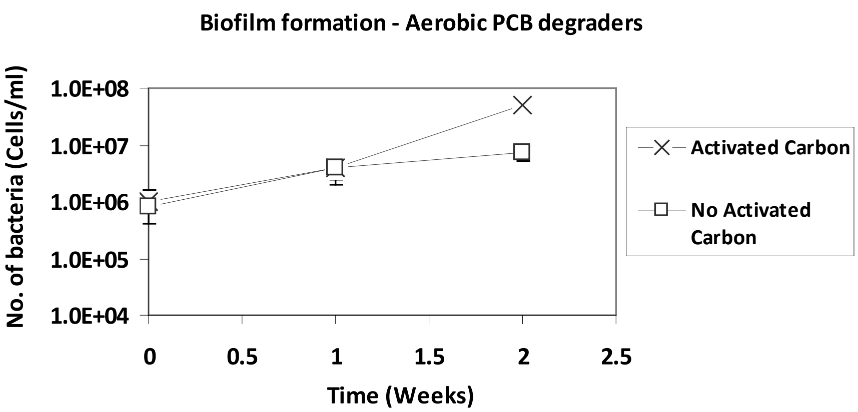

Figure 21. Biofilm formation of the aerobic PCB degrader Burkholderia xenovorans strain LB400 on activated carbon surfaces evaluated by DAPI staining.

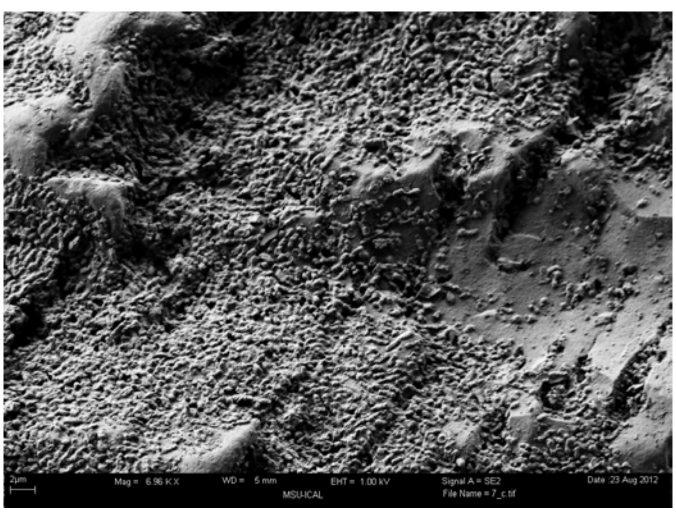

Figure 22. Biofilm formation of the aerobic PCB degrader Burkholderia xenovorans strain LB400 on activated carbon surfaces observed by SEM.

When the LB400 biofilm inoculum on activated carbon was applied to a sediment mesocosm as inoculum under similar conditions as the anaerobic biofilm delivery systems described above, degradation of A1248 was observed as well anaerobic dechlorination (Table 3). Since the sediment remained anaerobic in the mesocosms the indigenous dechlorinating bacteria continued the anaerobic processes, while *B. xenovorans* LB400 performed aerobic degradation, which has been observed in other projects as well (Tillmann, *et al.*, 2005). Since LB400 is a PCB degrader, LB400 associated enzymes are capable of opening the biphenyl ring structure and transform the molecule into a linear structure, this changed structure was not measured as a product in this experiment. However the results show lower increase in the congeners that contain 4 or less chlorines per biphenyl, which indicate that breakdown was taking place by LB400. Similar results have been seen in other sediment environments since it is known that LB400 can oxidize PCBs with up to 4-6 chlorine substitutions on the biphenyl rings (Bedard, *et al.*, 1986). This is particularly relevant for sediments contaminated with “lighter” Aroclor mixtures such as A1248, A1242 and on so.

Table 3. Activity of B. xenovorans LB400 biofilm covered activated carbon particles added as inoculum to sediment mesocosms compared to anaerobic DF1 biofilms.

Experiment	**Chlorines per biphenyl**		**Dechlorination rate**	**Increase ≤4 chlorines**
	Decrease	**In %**	**10^{-3} chlorines per biphenyl per day**	**Mol%**
DF1 Biofilm	1.4 ± 0.15	47.3	7.00 ± 0.74	10.2
LB400 Biofilm	1.2 ± 0.29	29.3	6.02 ± 1.47	7.2

<u>C5. Success criterion 3</u>

To show how a biofilm based delivery system can enhance PCB dechlorination rates and extent as well as influence PCB dechlorinating biofilm populations in mesocosms, when the biofilms are used as a delivery system for bioaugmentation.

The results obtained from the mesocosm study showed enhanced dechlorination (rates and extent), when the two biofilm based delivery systems were applied for bioaugmentation. The DF1 biofilm covering the activated carbon particles was investigated in detail to verify that biofilms formed on the activated carbon surfaces. The application of the advanced microscopic techniques showed that this was the case. Examination of the individual PCB congeners affected by the biofilm inoculum showed that the enriched sludge biofilm caused a wider range of congeners to dechlorinate, while simultaneously resulting in fewer dechlorination products compared to the DF1 biofilm. An analysis of the sediment showed that bacterial biofilms remained in the sediment, while the numbers and activities of the dechlorinating populations were maintained. This criterion for successful completion was therefore fulfilled.

In addition to the stated objectives for this project that focused on the anaerobic processes that make up the bottleneck for complete PCB removal, initial experiments investigating the biofilm formation by the aerobic degrader *Burkolderia xenovorans* LB400 and the effect in sediment were performed but not completed due to the time frame of the project. The results showed that LB400 is an efficient and fast biofilm former that readily adheres to activated carbon surfaces. In sediment the initial results indicate that the biofilm caused increased removal of less extensively chlorinated congeners, while the indigenous dechlorination was maintained. Further evaluations are needed, but the advantageous biofilm mode of growth was also shown by *B. xenovorans* LB400.

6. Conclusions and implications for future research

6.1 Overall conclusion

In this project application of biofilm based approaches were evaluated as microbial delivery systems. The dechlorinating biofilm covering activated carbon particles or located in the enriched sludge biofilm were applied as microbial inoculum systems to mesocosms consisting of PCB contaminated sediment. The results showed that both types of biofilm inoculum enhanced dechlorination of PCBs in the sediment mesocosms and that the dechlorinating bacteria remained in the sediment throughout the experiment and increased almost 2-fold in numbers. These results show that the proof of concept was documented.

The research in this project was performed with the objectives as well as the specific success criteria in mind and is discussed here:

Success criterion 1 was to establish inoculum consisting of anaerobically dechlorinating biofilms that can be used as a microbial inoculum delivery system in PCB contaminated sediment. For this purpose two biofilm approaches were examined: biofilm covered activated carbon particles and enriched sludge biofilm from wastewater. The results showed that it was possible to establish anaerobic biofilm with the dechlorinating organism DF1, that the bacteria attached and covered the activated carbon particles without disrupting the dechlorination process. In addition, the applied methods showed that dechlorinating bacteria were present in the enriched sludge biofilms and that the structure of the two types of biofilm were similar with respect to anaerobic dechlorination activity.

The second success criterion was to develop tools that could be used to analyze biofilms formed on a surface with regards to bacterial numbers and populations as well as PCB dechlorination activity. These new methods were predominantly based on fluorescent stains and advanced microscopy, since methods used for analysis of PCB dechlorination activity were established prior to this project. The microscopy techniques showed for the first time and in several ways the formation of biofilms on the activated carbon surfaces as well as the biofilm structure in the enriched sludge. The “tool box” for analyzing biofilms can subsequently be applied for other purposes not only with respect to anaerobic dechlorinating biofilms.

Success criterion 3 was to show how a biofilm based delivery system can enhance PCB dechlorination rates and extent as well as influence PCB dechlorinating biofilm populations in mesocosms, when the biofilms were used as a delivery system for bioaugmentation. The results showed that a wide range of congeners were dechlorinated, when the two biofilm based delivery systems were applied for bioaugmentation. This resulted in enhanced dechlorination rates. Enriched sludge biofilm augmentation caused dechlorination of a wider array of congeners due to the increased diversity of dechlorinating bacteria in the inoculum. Evaluation of the bacterial presence showed that the biofilm inoculum remained in the sediment throughout the experiment and in addition caused a 2-fold increase in the dechlorinating populations. Overall, the mesocosm experiments showed that application of a biofilm based delivery systems can enhance the dechlorination activity and that the bacteria remain in the environment.

In addition to the stated objectives for this project, experiments investigating the biofilm formation by the aerobic PCB degrader *Burkolderia xenovorans* LB400 and the effect in sediment were initiated. The results showed that *B. xenovorans* LB400 readily adhered to activated carbon surfaces and formed strong biofilms. In sediment, preliminary results indicated an increased removal of less extensively chlorinated congeners due to augmentation with *B. xenovorans* LB400 biofilm showing the advantageous biofilm mode of growth.

Overall, the results in this project showed that biofilms can play an important role in bioremediation of PCB contaminated sediments and that this altered way of approaching bioaugmentation by utilizing the way bacteria grow in nature can significantly benefit the clean-up strategies at contaminated sites.

<u>6.2 Next steps in follow-on research</u>

The next steps in a potential follow-on research project would be based on the positive results showing enhanced dechlorination and/or degradation of PCBs, when the biofilm inoculum was used as a delivery system. The anaerobic processes were in focus in this project and showed promising results, but also a continuation of the work with aerobic PCB degraders in order to complete the removal process, would be relevant in a follow-up project.

The overall goal for a potential follow-on research project would be to provide <u>biofilm based solutions</u> for bioaugmentation that would be tested under <u>environmental conditions</u> both under laboratory conditions but also under in situ conditions in the environment at a PCB contaminated site. The biofilm based solution has shown to be more robust and thus can withstand conditions that will be faced in the environment such as natural mixing, slow liquid exchange both horizontally and vertically, varying flow, tidal conditions that would expose the inoculum to a natural variation in aerobic versus anaerobic conditions. It is relevant to examine how biofilm based delivery systems would perform, when relevant environmental factors are taken into account.

The objectives for potential follow-on research would include, but not be limited to:

1. *Mixed aerobic and anaerobic biofilm delivery system*

Complete removal of PCBs from contaminated sites depend on initial anaerobic dechlorination to remove chlorines from extensively chlorinated congeners followed by aerobic degradation of the formed less chlorine congeners. A mixed biofilm associated with activated carbon or another sorptive surface would efficiently be able to perform both processes. In this project biofilm formation by both aerobic and anaerobic bacteria on activated was documented and showed that they separately took part in the removal of PCBs. In an earlier study of a heavily PCB contaminated site in Mechanicsburg, PA co-existence of aerobic PCB degraders and anaerobic PCB dechlorinating bacteria was found (Kjellerup, *et al.*, 2012). This showed that both types of activities have the potential to occur simultaneously, since the involved bacteria existed together in the environment.

Objective: To combine aerobic and anaerobic biofilms on activated carbon particles or on another type of delivery vehicle to supply the biofilm based inoculum that can complete PCB transformation to non-toxic forms simultaneously. The mixed biofilm would be made up by mixing cultures of bacteria with desirable anaerobic dechlorination characteristics with aerobic PCB degraders. Another possibility would be to benefit from natural sources such as wastewater sludge, where the inoculum contains a mixed bacterial population that already co-exist in sludge biofilms (see below).

2. Enriched biofilm originating from wastewater sludge as delivery system

In this project enhanced dechlorination of A1248 was occurring in sediment, where the biofilm inoculum originated from wastewater sludge due to the high numbers of dechlorinating bacteria, the diversity of the dechlorinating population and also the co-existence of the dechlorinating bacteria in the sludge biofilm. During wastewater treatment the sludge biofilm experiences aerobic conditions, where organic matter and ammonia in the wastewater is oxidized followed by anaerobic conditions where the oxidized compounds (nitrate, sulfate etc.) are reduced (Wu & Rodgers, 2010). Both aerobic and anaerobic bacteria are present in the sludge biofilm, but the organization in the biofilm matrix protects the anaerobic bacteria from oxygen exposure on the inside of the biofilm. Overall, the removal of PCBs follows the same pattern, where the anaerobic parts of the biofilm harbor the dechlorinating bacteria removing chlorines from the biphenyl structure, which leaves the PCB congeners with few chlorines attached, so they can be degraded aerobically by the aerobic degraders located in the outer parts of the biofilm. This co-existence is crucial for the nutrient removal requiring both aerobic and anaerobic conditions and the hypothesis is that the PCB transformation follows the same strategy.

Due to the nature of wastewater, where pathogenic bacteria are present, it is necessary to look into strategies for removing the risk for transfer of potential diseases. Currently, some wastewater treatment plants perform anaerobic digestion as a final process, methane is produced and where the temperature simultaneously is increased to 60-70C causing the pathogenic bacteria to die (Bertin, *et al.*, 2011). It would be possible to use the anaerobically digested sludge as inoculum if the dechlorination activity remains unaffected. Alternatively, it would be possible to enrich the very active dechlorinating cultures (as it was the case in this project) by using sterile sludge that would ensure the in situ conditions without the problem of pathogenic bacteria.

Objectives:

1. To enrich anaerobic sludge biofilms for active dechlorinating bacteria that can be utilized as inoculum in a delivery system and perform subsequent scale up.
2. To evaluate the wastewater sludge biofilms for presence of anaerobic dechlorinating bacteria and aerobic PCB degrading bacteria and evaluate their co-existence in the sludge biofilm. This would enable simultaneous anaerobic and aerobic process to occur thus promoting complete PCB mineralization and removal.
3. To examine the problem with presence of pathogenic bacteria in wastewater sludge in order to promote a safe solution for use of enriched sludge biofilms for bioaugmentation.
4. To establish a method for applying the enriched sludge biofilm to sediment to promote enhanced dechlorination and simultaneous aerobic degradation of PCBs, when oxygen is being supplied to create aerobic conditions.

3. Enhanced surface attachment and subsequent increased biofilm growth

In this project biofilms were shown to form both aerobically and anaerobically on a commercial form of activated carbon. However, one way of enhancing the dechlorination of PCBs in the sediment would be to increase the number of bacteria attaching to the activated carbon surfaces, so more bacteria would be in close proximity to the adsorbed PCBs on the activated carbon particles. The commercial activated carbon has not been evaluated for the potential for biofilm formation, so it is likely that other forms of activated carbon or other materials with high sorption capacity would be able to host increased biofilm growth thus enhancing the overall dechlorination process. Approaches for “anchoring” bacterial layers to the surfaces via enhanced initial attachment can be investigated thus ensuring that the initial attachment of bacteria will take place faster and thus promote a faster and more robust biofilm formation overall. Layers of proteins have been shown to promote the initial attachment of bacteria to surfaces (Ishida & Griffiths, 1999, Moscoso, *et al.*, 2006). An application would be to coat the activated carbon particles with a relevant mixture of proteins that also could benefit the subsequent bacterial growth and thus enhance the overall biofilm formation that can be applied as a delivery system for bioaugmentation.

Objective:

1. To examine different types of activated carbon as well as other kinds of sorptive materials in order to optimize the biofilm formation and increase the number of bacteria present in the biofilm.
2. To evaluate potential anchoring mechanisms (such as polysaccharides and proteins) together with optimization of growth conditions for biofilm formation to obtain enhanced biofilm delivery system.

4. Environmental and dynamic conditions

Mixed biofilm populations consisting of bacteria with different types of metabolic activities including both aerobic and anaerobic metabolic activities have been shown to be more robust and be able to withstand changing environmental conditions in the environment, since they are often hard to remove (Bohus, *et al.*, Zhang, *et al.*, 2007). Therefore, mixed biofilm populations with the capacity to anaerobically dechlorinate and aerobically degrade PCBs would also be more robust thus be able to withstand the varying environmental conditions taking place in PCB contaminated environments. These conditions could result from currents, tidal zones, weather exposure and would for instance cause mixing, varying surface flow and liquid exchange between different zones in the sediment. Existence in a biofilm embedded matrix would therefore protect the bacteria from these varying conditions and ensure that the PCB dechlorination as well as aerobic degradation activity would be sustained.

Objective:

To optimize and examine the robustness of mixed biofilm populations so enhanced dechlorination activity together with the subsequent aerobic PCB degradation can take place under dynamic environmental conditions. This would be done by performing experiments under laboratory conditions, where the conditions would imitate environmental conditions at the contaminated sites with regards to water flow and currents, tidal mixing etc. Afterwards, the obtained mixed biofilm delivery systems would be evaluated on site to observe the effect of the dynamic conditions occurring outside the laboratory.

Other

In the experiments in a follow-on project both spiked levels of PCBs as well as in situ low levels of contamination will be investigated depending on the objective of the specific experiment. In both situations, absolute values of the individual congeners will be measured in order to establish mass balances to follow the fate of the PCB congeners. This is particularly important, when aerobic degradation is being evaluated, since the degradation products from the break-down of the biphenyl ring structure (chlorobenzoic acids, benzoate) cannot be detected with the GC method used for analysis of chlorinated PCB congeners.

7. Presentations and publications

During the project period the research team has provided the required official project reports and presented research results at national and international symposia. Another important technology transfer mechanism in this project has to progressively pursue the education of undergraduate students at Goucher College. This transfer mechanism, although not immediate, is considered by organizations associated with this proposal as an extremely important component to our research. These students represent future technology users and more importantly, are the future professionals joining the environmental sciences and engineering community.

Several undergraduate students at Goucher College have worked on this project via summer research projects funded by SERDP (Summer of 2011) and funded by Goucher College (Summer of 2012). The summer research was presented at the 3rd and 4th Annual Landmark Summer Research Symposium taking place at Goucher College, Baltimore, MD in July 2011 and Moravian College, Bethlehem, PA July 2012.

The following students have been funded by the SERDP project ER-2135:

- Chiara Draghi (Research period: 2010-2013)
- Emily Balbier (Research period: 2011-2012)
- Freshta Akbari (Research period: 2013- ongoing)

7.1 Related publications

1. Kjellerup B.V, Naff C, Edwards S. J, Ghosh U, Baker J. E. Sowers, K. “Effects of activated carbon on reductive dechlorination of PCBs by halorespiring bacteria indigenous to sediments”. Water Research, September 2013. Under revision.
2. Demirtepe, H, Kjellerup B.V; Sowers K. and I. Imamoglu. “Modeling reductive PCB dechlorination activities in Baltimore Harbor sediment”. Submitted to Water Research, August 2013.
3. Edwards, S.J. and B.V. Kjellerup. “Applications of Biofilms in Bioremediation and Biotransformation of Persistent Organic Pollutants, Pharmaceuticals/Personal Care Products and Heavy Metals”. Applied Microbiology and Biotechnology, May 6, 2013. In Print. August 2013.
4. Kjellerup B.V, Paul P, Ghosh U, M, May H, Sowers, K. “Spatial distribution of PCB dechlorinating bacteria and activities in contaminated soil”. Applied and Environmental Soil Science. Volume 2012 (2012), Article ID 584970, doi:10.1155/2012/584970.
5. Kjellerup B.V, Stiell B, Baker, J, Sowers, K. “Horizontal and vertical distribution of anaerobic microbial dechlorination of PCBs, dechlorinating bacteria and activity in Baltimore Harbor, Maryland”. FEMS Microbial Ecology, 2013. In Prep
6. Sarah Edwards, Chiara Draghi, Kevin Sowers, B.V. Kjellerup. “Activated carbon particles covered with biofilms as a delivery vehicle for bioremediation of PCBs”.EST,2013. In Prep
7. Draghi, C, Edwards, S.J, Balbier, E, Ghosh and Kjellerup, B.V. “Activated sludge harbors the potential for important microbial PCB dechlorinating bacteria”. Water Research, 2013. In Prep
8. Chiara, Draghi, Sarah Edwards, Donna Fennell and Birthe Venø Kjellerup. “Review – The toxicity of PCB congeners involved in PCB dechlorination throughout the activated sludge wastewater process”. Chemosphere, 2013. In Prep

7.2 Invited Presentations citing SERDP funding

1. “Biofilm covered activated carbon particles enhance bioremediation of polychlorinated biphenyl (PCBs) in sediment”. 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies, June 2013, Jacksonville, Fl.
2. “Our slimy friends – Biofilms at work”, Seminar at Hood College, Frederick, MD, Department of Biology, March 19, 2013.
3. “Application of biofilm covered activated carbon particles as a microbial inoculum delivery system”, Presentation at SERDP In-Progress Review Meeting, Arlington, VA, Feb. 28, 2013.
4. “Application of biofilm covered activated carbon particles as a microbial inoculum delivery system for PCB bioremediation”, 6th ASM Conference on Biofilms, Miami, FL, Sept. 2012. Speaker.
5. “Application of biofilm covered activated carbon particles as a microbial inoculum delivery system”. Partners in Environmental Technology Symposium Workshop, Washington DC. Sediment Review Panel Meeting November 30, 2011. Speaker.
6. S. Edwards, C. Draghi and B.V. Kjellerup. “Biofilm covered activated carbon particles enhance bioremediation of polychlorinated biphenyl (PCBs) in sediment”. 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies, June 2013, Jacksonville, Fl.
7. S. Edwards, C. Draghi and B.V. Kjellerup. “Activated Carbon Facilitated Biofilm Development of the PCB degraders *Dehalobium chlorocoercia* DF1 and *Burkholderia xenovorans* strain LB400”. 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies, June 2013, Jacksonville, Fl.
8. Draghi, S. Edwards and B.V. Kjellerup. “Effect of anaerobic digestion and methane production on the presence of PCB dechlorinating bacteria, activity and toxicity in activated sludge”. 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies, June 2013, Jacksonville, Fl.
9. H. Demirtepe, B. V. Kjellerup, K. Sowers and I. Imamoglu. “Evaluation of the Major Anaerobic PCB Dechlorination Pathways in Baltimore Harbor Sediments”. 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies, June 2013, Jacksonville, Fl.
10. H. Demirtepe, B. V. Kjellerup, K. Sowers and I. Imamoglu. “Evaluation of PCB Dechlorination Pathways in Anaerobic Sediment Microcosms Using an Anaerobic Dechlorination Model”. 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies, June 2013, Jacksonville, Fl.
11. C. Draghi, S.J. Edwards, U. Ghosh and B. V. Kjellerup. “PCB metabolism in the Back River Waste Water Treatment Plant and identification of PCB degrading bacteria”. Environmental Health 2013, Boston, Ma.
12. S. J. Edwards, C. Draghi and B. V. Kjellerup. “Application of biofilm covered activated carbon particles as a microbial inoculum delivery system for PCB bioremediation”. 6th ASM Conference on Biofilms, September 2012, Miami, FL.
13. S. J. Edwards, C. Draghi and B. V. Kjellerup. “Activated Carbon Facilitated Biofilm Development and Metabolic Activity of Known PCB degraders, *Dehalobium chlorocoercia* DF1 and *Burkholderia xenovorans* strain LB400”. 6th ASM Conference on Biofilms, September 2012, Miami, FL.
14. S. J. Edwards, C. Draghi and B. V. Kjellerup. “Bacterial biofilm communities enhance the

degradation of polychlorinated biphenyls (PCBs) in sediment”, 14^{th} International

15. S. Edwards, K. Sowers and B.V. Kjellerup. “Biofilm Growth of the Dechlorinating Bacteria, DF1, on activated carbon for PCB degradation”. ASM MD Branch Student Symposium 2012, Baltimore, MD, May 2012Symposium on Microbial Ecology, Copenhagen, Denmark, August 2012.
16. C. Draghi and B. V. Kjellerup. “Degradation of PCBs (Aroclor 1248) in biofilms consisting of *Burkholderia xenovorans* strain LB400 covering activated carbon particles”. ASM MD Branch Student Symposium 2012, Baltimore, MD, May 2012.
17. S.J. Edwards, A. Houston, C. Draghi, E. Balbier and B.V. Kjellerup. “The Biofilm Tree of Life: An Example of Dr. Costerton’s influence”. Festschrift honoring Dr. Bill Costerton, Pittsburgh, PA, Feb. 2012.
18. S.J. Edwards, C. Draghi, E. Balbier and B.V. Kjellerup. “Application of biofilm covered activated carbon particles as a microbial inoculum delivery system for PCB bioremediation”. Partners in Environmental Technology Symposium Workshop, Washington DC, Dec. 2011.
19. C. Draghi, K.R. Sowers, B.V. Kjellerup. “Biofilm Formation On Activated Carbon Particles Enhances PCB Dechlorinating Activity In Sediment”. ASM 111^{th} General Meeting, New Orleans, LA, May 2011

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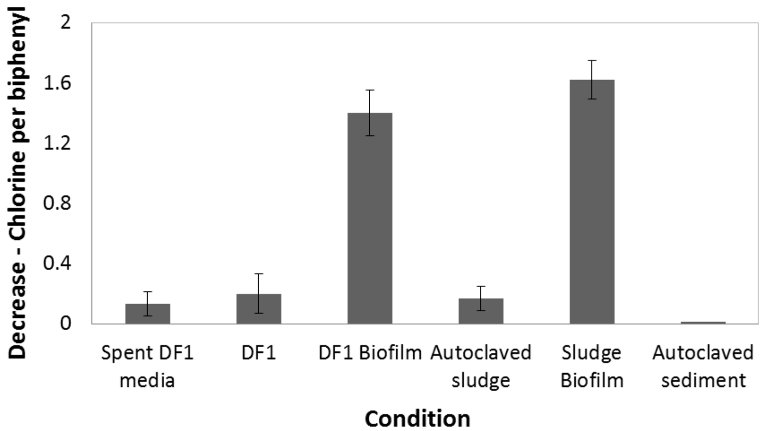
Appendix 1

In this appendix the backup date for the figures: 12, 13, and 16 are included. The backup date for figures 14 and 15 are placed in Appendix 2 (horizontal format). No additional data are included for Figure 17, since the chromatograms are the direct output from the DHPLC analysis.

The original figures and figure legends have also been included in order to clearly indicate, which graph the data support.

Figure 12. The decrease in chlorines per biphenyl in the mesocosm experiment involving anaerobic conditions with DF1 biofilm as well as enriched sludge biofilm.

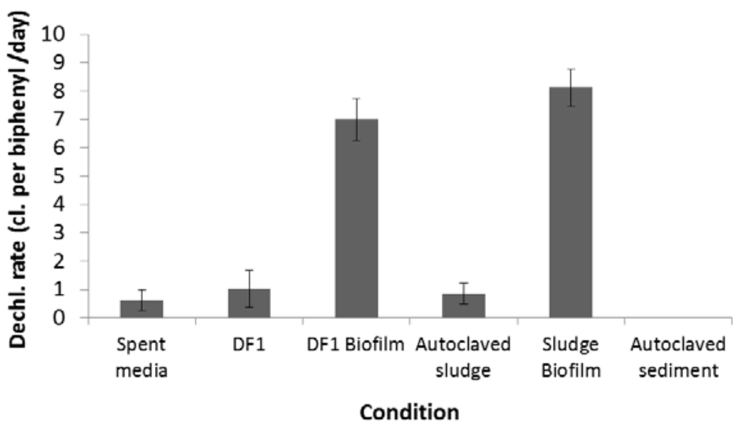


The decrease in chlorines per biphenyl was calculated by calculating the relative number of chlorines in a homolog group by adding the sum of the measured masses of all congeners in a homolog group and multiplying by the number of chlorines and subsequently divide by the sum of all relative chlorine numbers. The data obtained from each of the three replicates were averaged and the standard deviation was calculated based on this. The specific decrease for a mesocosm over the 200 day period was compared to the control with sediment spiked with Aroclor 1248 by subtracting the two numbers.

Calculation of chlorines per biphenyl in sediment			
No. cl	**Homolog**	**Amount (μg)**	**Cl per biphenyl**
1	Mono	0.00E+00	0.00E+00
2	Di	3.21E+00	6.42E+00
3	Tri	6.01E+00	1.80E+01
4	Tetra	8.37E+00	3.35E+01
5	Penta	6.50E+00	3.25E+01
6	Hexa	1.63E+00	9.77E+00
7	Hepta	2.00E-02	1.40E-01
8	Octa	0.00E+00	1.60E-01
9	Nona	0.00E+00	0.00E+00
10	Deca	0.00E+00	0.00E+00
	SUM	**25.73274**	**3.90E+00**
Chlorines per biphenyl			**3.90E+00**

Decrease in chlorines per biphenyl compared to sediment (Chlorines per biphenyl)				
		Average		Standard Deviation
Exp. no	**Condition**	**Decrease**	**In%**	**Decrease**
3	Spent DF1 media	0.13	5	0.08
5	DF1 liquid	0.2	5.2	0.13
8	DF1 Biofilm on GAC	1.4	47.3	0.15
13	Autoclaved sludge	0.17	4.3	0.08
12	Sludge Biofilm	1.62	73.2	0.13
14	Autoclaved sediment	0.011	0.3	0

Figure 13. The dechlorination rates for anaerobic activity in the mesocosm experiment involving anaerobic conditions with DF1 biofilm as well as enriched activated sludge biofilm.



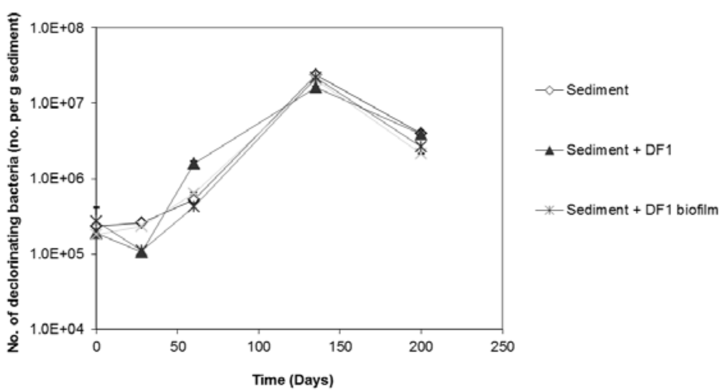
To get the dechlorination rates in mMol%, the mass of the individual congeners was measured on the GC in the 1 ml extracts. This congener mass was converted to mass percent by dividing the mass of the congener by the total mass of all detected congeners. This mass percent was then divided by the molecular weight of the specific congener in order to obtain the relative molar presence. The molar percent was subsequently obtained by dividing the relative presence of the specific congener by the sum of molar presence for all detected congeners.

No. cl	Homolog	Amount	Mass %	Mol%
1	Mono	7.91E+00	2.37E-01	30.18
2	Di	1.09E+01	3.26E-01	35.03
3	Tri	2.36E+00	7.07E-02	6.76
4	Tetra	5.46E+00	1.64E-01	13.45
5	Penta	4.41E+00	1.32E-01	9.84
6	Hexa	2.26E+00	6.79E-02	4.59
7	Hepta	8.53E-02	2.56E-03	0.16
8	Octa	0.00E+00	0.00E+00	0.00
9	Nona	0.00E+00	0.00E+00	0.00
10	Deca	0.00E+00	0.00E+00	0.00
	SUM	**33.35162**	**1.00E+00**	**100.00**

The dechlorination rate in mmol% per day was found by calculating the change in mol% between day 0 and day 200 and subsequently divide this by 200 days in which the experiment took place.

Dechlorination rate (mMol% per day)			
Exp. no	**Condition**	**Average**	**Standard Deviation**
3	Spent media	0.62	0.37
5	DF1	1.02	0.66
8	DF1 Biofilm	7	0.74
13	Autoclaved sludge	0.85	0.37
12	Sludge Biofilm	8.12	0.65
14	Autoclaved sediment	0	0

Figure 16. The number of dechlorinating bacteria in mesocosm samples inoculated with anaerobic cultures of DF1 and enriched sludge biofilms together with the relevant controls over the course of the experiment.



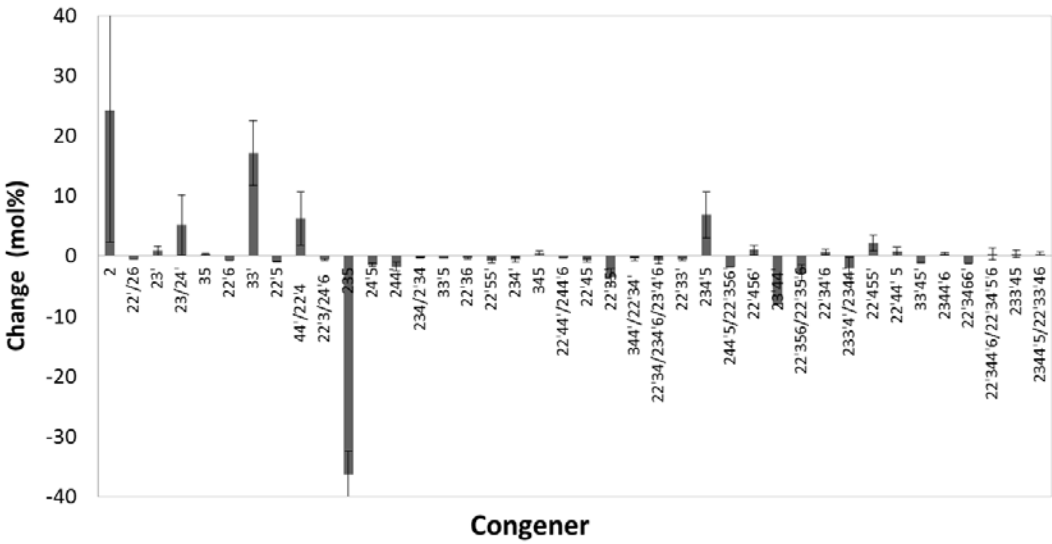
Number of dechlorinating bacteria determined by Q-PCR in sediment mesocosms (cells per g sediment)									
				Average				***Standard Deviation***	
Exp no.	Mesocosm/Sampling Day	**0**	**28**	**60**	**135**	**200**	***60***	***135***	***200***
1	Sediment + A1248	1.65E+02	2.59E+05	5.18E+05	2.38E+07	3.92E+06	2.76E+04	2.74E+05	7.02E+05
2	Sediment	3.31E+05	1.01E+05	5.01E+05	2.14E+07	4.11E+06	1.52E+05	6.78E+05	4.93E+05
5	Sediment + DF1	1.88E+05	1.06E+05	1.57E+06	1.60E+07	3.89E+06	1.20E+05	6.72E+05	2.86E+05
7	Sediment + DF1 & GAC individually	1.80E+05	2.34E+05	6.34E+05	2.01E+07	2.12E+06	2.73E+04	9.53E+05	2.99E+04
8	Sediment + DF1 biofilm	2.70E+05	1.11E+05	4.25E+05	2.15E+07	2.66E+06	3.77E+04	2.21E+06	1.09E+05
12	Sediment + sludge enrich	1.90E+05	3.65E+05			2.80E+06			5.58E+05
13	Sediment + autoclaved sludge	1.79E+02	1.54E+02		3.58E+06	2.88E+05		4.66E+05	6.86E+03
14	Autoclaved sediment	9.48E+01	1.09E+03	1.80E+01		1.08E+03	1.43E+01		

Appendix 2

In this appendix the backup date for the figures: 14 and 15 are included. The backup date for the figures 12, 13, 16 and 17 are placed in Appendix 1 (Portrait format). The original figures and figure legends have also been included in order to clearly indicate, which graph the data support. The explanation of data fields and units are described below for both figures.

First row of data tables in for Figure 14 and 15:													
Name	D0	D200a	D200b	D200c	D200A-D0	D200B-D0	D200C-D0	D200-D0 AV	STDEV	Configuration	Configuration [>0.25 mol% change]	D200-D0 AV	STDEV
Explanation of the data fields:													
Congener name	Time point (average D0)	Time point (replicate A)	Time point (replicate B)	Time point (replicate C)	The mol% of the congener at D0 subtracted from the mol% of the same congener at D200	The mol% of the congener at D0 subtracted from the mol% of the same congener at D200	The mol% of the congener at D0 subtracted from the mol% of the same congener at D200	Average of the D200-D0 difference	Standard deviation of the D200-D0 difference	Configuration of the congeners. All included in the analysis	Configuration of the congeners. Included in Figure 14 (>0.25 mol% change).	Average of the D200-D0 difference. Included in Figure 14 (>0.25 mol% change).	Standard deviation of the D200-D0 difference. Included in Figure 14 (>0.25 mol% change).
Units:													
-	Mol%	Mol%	Mol%	Mol%	Mol%	Mol%	Mol%	Mol%	Mol%	-	-	Mol%	Mol%

Figure 14. Change in individual congeners in the mesocosm inoculated with anaerobic biofilm covered activated carbon particles. Congeners with a change >0.25 mol% were included.



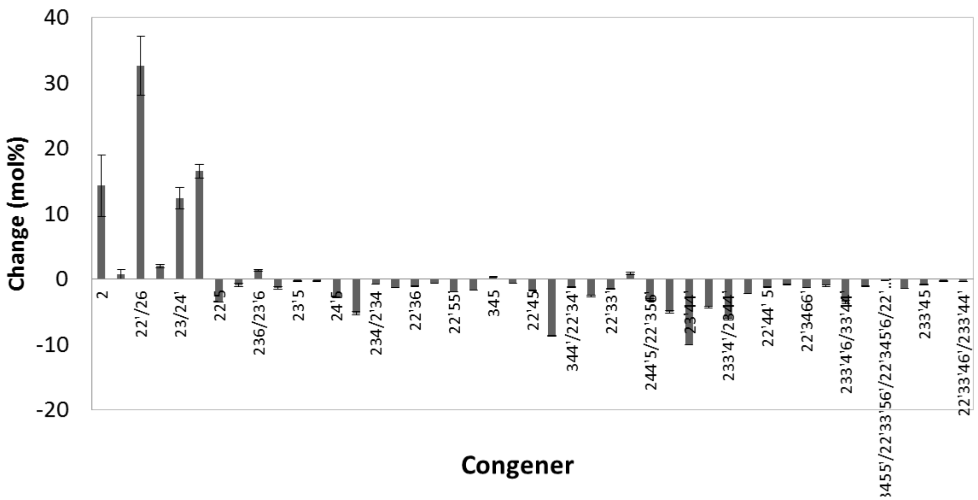
Name	D0 AV	D200a	D200b	D200c	D200A-D0	D200B-D0	D200C-D0	D200-D0 AV	STDEV	Configuration	Configuration	D200-D0 AV	STDEV
										ALL	>0.25 mol% change		
C1	0	30.17609	42.5517	0	30.17609	42.5517	0	24.2426	21.88759	2	2	24.2425956	21.88759
3	0	0	0	0	0	0	0	0	0	4	22'/26	-0.53539849	0
C4,10	0.535398	0	0	0	-0.5354	-0.5354	-0.5354	-0.5354	0	22' 26	23'	0.84263027	0.830839
C7,9	0.124613	0	0.433757	0	-0.12461	0.309143	-0.12461	0.019972	0.25043	24 25	23/24'	5.14363469	4.955514
C6	0.110625	1.523539	0	1.336227	1.412914	-0.11063	1.225602	0.84263	0.830839	23'	35	0.31793539	0.148677
C5,8	0	9.886716	5.544188	0	9.886716	5.544188	0	5.143635	4.955514	23 24'	22'6	-0.75245802	1.36E-16
C14	0	0.257835	0.487255	0.208717	0.257835	0.487255	0.208717	0.317935	0.148677	35	33'	17.1629967	5.364606
C19	0.752458	0	0	0	-0.75246	-0.75246	-0.75246	-0.75246	1.36E-16	22'6	22'5	-0.95693056	0.076868
C11	0	23.35726	14.01811	14.11361	23.35726	14.01811	14.11361	17.163	5.364606	33'	44'/22'4	6.20368442	4.466436
12, 13	0	0	0	0	0	0	0	0	0	34, 34'	22'3/24'6	-0.61829845	0.147738
C18	1.001311	0	0	0.13314	-1.00131	-1.00131	-0.86817	-0.95693	0.076868	22'5	235	-36.3166529	3.934061
C15,17	0.44842	2.696353	5.764133	11.49583	2.247933	5.315713	11.04741	6.203684	4.466436	44' 22'4	24'5	-1.44111151	0.304694
C24,27	0.048906	0.077655	0	0	0.028749	-0.04891	-0.04891	-0.02302	0.044834	236 23'6	244'	-1.79541756	0.748082
C16,32	0.874378	0.14642	0.197738	0.424081	-0.72796	-0.67664	-0.4503	-0.6183	0.147738	22'3 24'6	234/2'34	-0.27427251	0.040681
23	40.84215	2.438472	2.07471	9.063299	-38.4037	-38.7674	-31.7788	-36.3167	3.934061	235	33'5	-0.36955765	0.055626
29	0	0	0.049111	0.075551	0	0.049111	0.075551	0.041554	0.038338	245	22'36	-0.33358889	0.096402
C54	0	0	0	0	0	0	0	0	0	22'66'	22'55'	-0.7844106	0.384755
C26	0.274503	0.134028	0	0.285274	-0.14048	-0.2745	0.010771	-0.13474	0.142724	23'5	234'	-0.57252474	0.393284
C25	0.117729	0.105506	0	0.221184	-0.01222	-0.11773	0.103455	-0.00883	0.110631	23'4	345	0.4743971	0.382444
C31	1.679513	0.133522	0	0.581682	-1.54599	-1.67951	-1.09783	-1.44111	0.304694	24'5	22'44'/244'6	-0.35868963	6.8E-17
50	0	0	0	0	0	0	0	0	0	22'46	22'45	-0.68244317	0.309624

28	2.478318	0.26381	0.238306	1.546586	-2.21451	-2.24001	-0.93173	-1.79542	0.748082	244'	22'35'	-3.54840982	1.45843
C21,33	0.314022	0	0.037945	0.081303	-0.31402	-0.27608	-0.23272	-0.27427	0.040681	234 2'34	344'/22'34'	-0.46257435	0.231228
53	0.191497	0.119136	0	0.086557	-0.07236	-0.1915	-0.10494	-0.12293	0.061572	22'56'	22'34/234'6/23'4'6	-0.6581302	0.590098
C51	0.04939	0	0	0.051166	-0.04939	-0.04939	0.001776	-0.03233	0.029541	22'46'	22'33'	-0.54605969	0.195613
	0.595801	0.200901	0.1878	0.290028	-0.3949	-0.408	-0.30577	-0.36956	0.055626	33'5	234'5	6.84538234	3.835374
	0.555744	0.112022	0.291237	0.263206	-0.44372	-0.26451	-0.29254	-0.33359	0.096402	22'36	244'5/22'356'	-1.77398631	2.72E-16
	0	0	0.210761	0.094685	0	0.210761	0.094685	0.101815	0.105561	34'5	22'456'	0.99057133	0.820496
C46	0.317997	0	0.192383	0.195092	-0.318	-0.12561	-0.1229	-0.18884	0.111863	22'36'	23'44'	-7.94022953	0
52,	1.197535	0.18044	0.201702	0.857232	-1.0171	-0.99583	-0.3403	-0.78441	0.384755	22'55'	22'356/22'35'6	-2.76753875	1.284158
C73	0	0	0	0	0	0	0	0	0	23'5'6	22'34'6	0.62417552	0.430545
22	1.073243	0.292461	0.255357	0.954339	-0.78078	-0.81789	-0.1189	-0.57252	0.393284	234'	233'4'/2344'	-1.96504285	1.717659
C49	0	0	0	0	0	0	0	0	0	22'45'	22'455'	2.11911075	1.321228
38	0	0.274767	0.233076	0.915348	0.274767	0.233076	0.915348	0.474397	0.382444	345	22'44' 5	0.74750873	0.743131
C47,75	0.35869	0	0	0	-0.35869	-0.35869	-0.35869	-0.35869	6.8E-17	22'44' 244'6	33'45'	-1.1161141	0.033371
C48	1.109942	0.223369	0.275367	0.783759	-0.88657	-0.83457	-0.32618	-0.68244	0.309624	22'45	2344'6	0.40452834	0.181077
65	0	0	0	0	0	0	0	0	0	2356	22'3466'	-1.25455689	0
C62	0	0.019386	0.058163	0	0.019386	0.058163	0	0.02585	0.029615	2346	22'344'6/22'34'5'6	0.38422906	1.041925
C35	0	0	0	0	0	0	0	0	0	33'4	233'45	0.36899936	0.610658
104	0	0	0	0.102363	0	0	0.102363	0.034121	0.059099	22'466'	2344'5/22'33'46	0.35015698	0.367268
C44	5.114104	0.722256	0.725083	3.249743	-4.39185	-4.38902	-1.86436	-3.54841	1.45843	22'35'			
C37,42	0.754843	0.173997	0.1441	0.558709	-0.58085	-0.61074	-0.19613	-0.46257	0.231228	344' 22'34'			
72	0.013339	0	0.032809	0	-0.01334	0.01947	-0.01334	-0.0024	0.018942	23'55'			
68	0	0	0	0	0	0	0	0	0	23'45'			
C41,64,71	1.742851	0.690019	0.801059	1.763085	-1.05283	-0.94179	0.020234	-0.65813	0.590098	22'34 234'6 23'4'6			
C103	0	0	0	0	0	0	0	0	0	22'45'6			
57	0	0	0	0	0	0	0	0	0	233'5			
40	0.929509	0.164632	0.444339	0.541376	-0.76488	-0.48517	-0.38813	-0.54606	0.195613	22'33'			
C67,100	0.073318	0.043858	0.068927	0.092755	-0.02946	-0.00439	0.019437	-0.0048	0.024451	23'45 22'44'6			
C63	0.414943	3.756497	6.666502	11.35798	3.341554	6.251559	10.94303	6.845382	3.835374	234'5			
C74,94	1.773986	0	0	0	-1.77399	-1.77399	-1.77399	-1.77399	2.72E-16	244'5 22'356'			
61	0	0	0	0	0	0	0	0	0	2345			
C70	5.416807	5.398156	3.196133	7.147598	-0.01865	-2.22067	1.730791	-0.16951	1.980047	23'4'5			
98	0	0	0	0	0	0	0	0	0	22'346			
C102	0	0.688277	0.364108	1.919329	0.688277	0.364108	1.919329	0.990571	0.820496	22'456'			
66	7.94023	0	0	0	-7.94023	-7.94023	-7.94023	-7.94023	0	23'44'			
93, 95	4.519558	1.107057	0.918178	3.230822	-3.4125	-3.60138	-1.28874	-2.76754	1.284158	22'356 22'35'6			
91	0	0.575375	0.22011	1.077042	0.575375	0.22011	1.077042	0.624176	0.430545	22'34'6			
C55	0.182265	0	0	0	-0.18227	-0.18227	-0.18227	-0.18227	0	233'4			
C56,C60	4.375235	1.82792	1.059356	4.343301	-2.54732	-3.31588	-0.03193	-1.96504	1.717659	233'4' 2344'			

101	0	1.6232	1.117586	3.616546	1.6232	1.117586	3.616546	2.119111	1.321228	22'455'			
C90	0	0	0	0	0	0	0	0	0	22'34'5			
113	0.102928	0.028746	0.046889	0.061602	-0.07418	-0.05604	-0.04133	-0.05718	0.016458	233'5'6			
99	0	0.576858	0.104546	1.561122	0.576858	0.104546	1.561122	0.747509	0.743131	22'44' 5			
79	1.135381	0.0578	0	0	-1.07758	-1.13538	-1.13538	-1.11611	0.033371	33'45'			
112	0.06784	0	0	0.057585	-0.06784	-0.06784	-0.01026	-0.04865	0.033247	233'56			
C78,83	0.271408	0.218221	0.208296	0.284479	-0.05319	-0.06311	0.013071	-0.03441	0.041417	33'45 22'33'5			
C97	0.900046	0.650358	0.705078	1.153816	-0.24969	-0.19497	0.253771	-0.06363	0.276234	22'3'45			
86	0.063735	0.035519	0	0.056913	-0.02822	-0.06373	-0.00682	-0.03292	0.028747	22'345			
C81,87	0	0	0.124642	0.168369	0	0.124642	0.168369	0.09767	0.087365	344'5 22'345'			
115	0	0.376932	0.238834	0.59782	0.376932	0.238834	0.59782	0.404528	0.181077	2344'6			
145	1.254557	0	0	0	-1.25456	-1.25456	-1.25456	-1.25456	0	22'3466'			
120	0	0	0	0	0	0	0	0	0	23'455'			
85	1.046574	0.872438	0.788276	1.533495	-0.17414	-0.2583	0.486922	0.018163	0.408132	22'344'			
C136	0	0	0	0	0	0	0	0	0	22'33'66'			
C110,77	2.890675	2.117967	1.616779	4.34271	-0.77271	-1.2739	1.452035	-0.19819	1.450941	233'4'6 33'44'			
C151	0.777312	0.798711	0.882902	1.264409	0.0214	0.10559	0.487097	0.204696	0.248163	22'355'6			
C124,135,144, 147	0.197446	0.314909	0.5242	0.453007	0.117463	0.326754	0.255562	0.233259	0.106413	2'3455' 22'33'56' 22'345'6 22'34'56			
C107,108	0	0.145882	0	0.266521	0.145882	0	0.266521	0.137468	0.13346	233'4'5 233'45'			
139, 149	0.754853	1.37325	2.043997	0	0.618397	1.289144	-0.75485	0.384229	1.041925	22'344'6 22'34'5'6			
106	0.701197	0.782279	0.656722	1.771588	0.081083	-0.04448	1.070391	0.368999	0.610658	233'45			
133	0	0	0	0.023963	0	0	0.023963	0.007988	0.013835	22'33'55'			
C134	0	0	0	0	0	0	0	0	0	22'33'56			
C114,131	0.321509	0.546672	0.383209	1.085116	0.225163	0.061701	0.763607	0.350157	0.367268	2344'5 22'33'46			
C165	0	0.009715	0.010823	0.016762	0.009715	0.010823	0.016762	0.012433	0.00379	233'55'6			
C146	0.085775	0.161161	0.151771	0.220224	0.075387	0.065996	0.134449	0.091944	0.037109	22'34'55'			
C161	0	0	0	0	0	0	0	0	0	233'45'6			
184	0	0	0	0	0	0	0	0	0	22'344'66'			
153	0.278587	0.449223	0.324163	0.818486	0.170636	0.045576	0.5399	0.252037	0.257019	22'44'55'	22'44'55'	0.25203718	0.257019
168	0	0	0	0	0	0	0	0	0	23'44'5'6			
127	0	0	0	0	0	0	0	0	0	33'455'			
132, 105	0	0.369654	0.193606	0.614353	0.369654	0.193606	0.614353	0.392538	0.211305	22'33'46' 233'44'	22'33'46' 233'44'	0.39253783	0.211305
179	0.366867	0.155216	0.085551	0.055192	-0.21165	-0.28132	-0.31167	-0.26821	0.051283	22'33'566'	22'33'566'	-0.26821352	0.051283
141	0	0.281398	0	0.189374	0.281398	0	0.189374	0.156924	0.143478	22'3455'			
137, 176, 130	0.02026	0.066898	0.040789	0.036628	0.046638	0.020529	0.016368	0.027845	0.016408	22'344'5 22'33'466' 22'33'45'			
164	0	0.026374	0	0.030736	0.026374	0	0.030736	0.019037	0.01663	233'4'5'6			
163, 138	0.098739	0.192887	0.120823	0.27719	0.094148	0.022084	0.178451	0.098228	0.078263	233'4'56			

										22344'5'			
C158	0	0	0	0	0	0	0	0	0	233'44'6			
160	0.021031	0	0.069831	0	-0.02103	0.0488	-0.02103	0.002246	0.040317	234'456			
186	0	0	0	0	0	0	0	0	0	22'34566'			
C126,129,178	0.025496	0	0.102272	0	-0.0255	0.076776	-0.0255	0.008595	0.059047	33'44'5 22'33'45 22'33'55'6			
C175	0	0	0	0	0	0	0	0	0	22'33'45'6			
159	0	0	0	0	0	0	0	0	0	233'455'			
186, 182	0.049139	0	0.080619	0	-0.04914	0.03148	-0.04914	-0.02227	0.046546	22'34566' 22'344'56'			
C183	0.050785	0	0.280128	0	-0.05078	0.229344	-0.05078	0.042591	0.161732	22'344'5'6			
C167	0	0	0	0	0	0	0	0	0	23'44'55'			
C128	0.05372	0	0.160908	0	-0.05372	0.107187	-0.05372	-8.4E-05	0.0929	22'33'44'			
185	0	0	0	0	0	0	0	0	0	22'3455'6			
C174	0	0	0.083133	0	0	0.083133	0	0.027711	0.047997	22'33'456'			
181	0.003953	0	0	0	-0.00395	-0.00395	-0.00395	-0.00395	0	22'344'56			
C177	0.026773	0	0.096039	0	-0.02677	0.069265	-0.02677	0.00524	0.055448	22'33'4'56			
202, 171	0	0	0.013621	0	0	0.013621	0	0.00454	0.007864	22'33'55'66' 22'33'44'6			
	0.091367	0	0.218391	0	-0.09137	0.127024	-0.09137	-0.01857	0.126088	233'44'5			
C173	0	0	0	0	0	0	0	0	0	22'33'456			
C197	0	0	0	0	0	0	0	0	0	22'33'44'66'			
C192	0	0	0	0	0	0	0	0	0	233'455'6			
C180	0.017458	0	0.040065	0	-0.01746	0.022606	-0.01746	-0.0041	0.023131	22'344'55'			
C193	0	0	0	0	0	0	0	0	0	233'4'55'6			
C191	0	0	0	0	0	0	0	0	0	233'44'5'6			
C199	0	0	0	0	0	0	0	0	0	22'33'4566'			
169	0	0	0	0	0	0	0	0	0	33'44'55'			
C170,190	0.012258	0	0.074671	0	-0.01226	0.062414	-0.01226	0.012633	0.043112	22'33'44'5 233'44'56			
198	0	0	0.017602	0	0	0.017602	0	0.005867	0.010162	22'33'455'6			
201	0	0	0.169854	0	0	0.169854	0	0.056618	0.098065	22'33'45'66'			
C196,203	0	0	0.087759	0	0	0.087759	0	0.029253	0.050667	22'344'55'6 22'33'44'56'			
C195,208	0	0	0.020252	0	0	0.020252	0	0.006751	0.011693	22'33'455'66' 22'33'44'56			
C207	0	0	0	0	0	0	0	0	0	22'33'44'566'			
C194	0	0	0.021979	0	0	0.021979	0	0.007326	0.01269	22'33'44'55'			
C205	0	0	0	0	0	0	0	0	0	233'44'55'6'			
C206	0	0	0.02036	0	0	0.02036	0	0.006787	0.011755	22'33'44'55'6			
C209	0.009168	0	0.129531	0	-0.00917	0.120362	-0.00917	0.034009	0.074785	22'33'44'55'66'			

Figure 15. Change in individual congeners in the mesocosm inoculated with anaerobic biofilm enriched from wastewater sludge. Congeners with a change >0.25 mol% were included.



Name	D0	D200a	D200b	D200c	D200A-D0	D200B-D0	D200C-D0	D200-D0 AV	STDEV	Configuration	Configuration	D200-D0 AV	STDEV
										ALL	>0.25 mol% change		
C1	0.00000	11.53807	19.77487	11.56606	11.53807	19.77487	11.56606	14.29300	4.74746	2	2	14.293	4.747462
3	0.00000	1.31431	0.00000	0.95766	1.31431	0.00000	0.95766	0.75732	0.67967	4	4	0.757325	0.679672
C4,10	0.00000	37.85130	29.59093	30.44895	37.85130	29.59093	30.44895	32.63039	4.54175	22' 26	22'/26	32.63039	4.541748
C7,9	0.36998	0.71149	0.54320	0.46764	0.34151	0.17322	0.09766	0.20413	0.12483	24 25	23'	2.011561	0.301873
C6	0.00000	1.69575	2.29724	2.04169	1.69575	2.29724	2.04169	2.01156	0.30187	23'	23/24'	12.38885	1.603474
C5,8	5.73903	16.33830	18.61134	19.43400	10.59927	12.87230	13.69496	12.38885	1.60347	23 24'	22'6	16.51534	1.051382
C14	0.07821	0.00000	0.00000	0.00000	-0.07821	-0.07821	-0.07821	-0.07821	0.00000	35	22'5	-3.45941	0.012629
C19	3.65375	21.26667	19.17097	20.06964	17.61291	15.51722	16.41589	16.51534	1.05138	22'6	44'/22'4	-0.91114	0.25705
C11	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	33'	236/23'6	1.357447	0.136099
12, 13	0.00000	0.00000	0.00000	0.06336	0.00000	0.00000	0.06336	0.02112	0.03658	34, 34'	22'3/24'6	-1.33921	0.25264
C18	3.61741	0.15877	0.17024	0.14502	-3.45864	-3.44717	-3.47240	-3.45941	0.01263	22'5	23'5	-0.28939	0.023765
C15,17	1.45784	0.57827	0.27533	0.78652	-0.87957	-1.18251	-0.67132	-0.91114	0.25705	44' 22'4	23'4	-0.29638	0.056355
C24,27	0.30586	1.78967	1.51921	1.68105	1.48381	1.21335	1.37518	1.35745	0.13610	236 23'6	24'5	-2.79362	0.020899
C16,32	3.05942	1.50071	1.66354	1.99637	-1.55870	-1.39588	-1.06305	-1.33921	0.25264	22'3 24'6	244'	-5.2106	0.265151
23	0.15391	0.01462	0.00000	0.02863	-0.13929	-0.15391	-0.12528	-0.13949	0.01431	235	234/2'34	-0.72193	0

29	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	245	33'5	-1.32995	0.014249
C54	0.00000	0.01818	0.00000	0.02762	0.01818	0.00000	0.02762	0.01527	0.01404	22'66'	22'36	-1.10118	0.022801
C26	0.73596	0.42084	0.46771	0.45115	-0.31512	-0.26826	-0.28481	-0.28939	0.02377	23'5	22'36'	-0.6125	0.018855
C25	0.38726	0.03561	0.08875	0.14826	-0.35165	-0.29851	-0.23900	-0.29638	0.05636	23'4	22'55'	-1.96489	0.013512
C31	3.77669	0.98190	1.00453	0.96278	-2.79479	-2.77216	-2.81390	-2.79362	0.02090	24'5	234'	-1.67687	0.025993
50	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'46	345	0.327409	0.082328
28	5.59246	0.17792	0.28606	0.68160	-5.41454	-5.30639	-4.91086	-5.21060	0.26515	244'	22'44'/244'6	-0.59804	0
C21,33	0.72193	0.00000	0.00000	0.00000	-0.72193	-0.72193	-0.72193	-0.72193	0.00000	234 2'34	22'45	-1.77427	0.059851
53	0.00000	0.06740	0.05200	0.08049	0.06740	0.05200	0.08049	0.06663	0.01426	22'56'	22'35'	-8.62338	0.054667
C51	0.09373	0.03327	0.03203	0.02859	-0.06046	-0.06170	-0.06514	-0.06243	0.00242	22'46'	344'/22'34'	-1.20877	0.012572
	1.36071	0.02159	0.04717	0.02350	-1.33911	-1.31354	-1.33721	-1.32995	0.01425	33'5	22'34/234'6/23'4'6	-2.57035	0.097041
	1.12616	0.04468	0.00000	0.03024	-1.08148	-1.12616	-1.09591	-1.10118	0.02280	22'36	22'33'	-1.4937	0.089042
	0.00000	0.00000	0.00000	0.05365	0.00000	0.00000	0.05365	0.01788	0.03098	34'5	234'5	0.876147	0.191141
C46	0.66540	0.03298	0.05528	0.07046	-0.63243	-0.61012	-0.59494	-0.61250	0.01886	22'36'	244'5/22'356'	-3.1204	0.148426
52,	2.03332	0.05671	0.06537	0.08321	-1.97661	-1.96795	-1.95011	-1.96489	0.01351	22'55'	23'4'5	-4.98282	0.163272
C73	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	23'5'6	23'44'	-10.0802	0
22	1.76408	0.07485	0.06972	0.11709	-1.68923	-1.69437	-1.64700	-1.67687	0.02599	234'	22'356/22'35'6	-4.26419	0.135953
C49	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'45'	233'4'/2344'	-6.10351	0.168799
38	0.00000	0.30222	0.26062	0.41939	0.30222	0.26062	0.41939	0.32741	0.08233	345	22'34'5	-2.13301	0
C47,75	0.59804	0.00000	0.00000	0.00000	-0.59804	-0.59804	-0.59804	-0.59804	0.00000	22'44' 244'6	22'44' 5	-1.23955	0.08692
C48	1.80883	0.00000	0.00000	0.10366	-1.80883	-1.80883	-1.70516	-1.77427	0.05985	22'45	22'3'45	-0.77716	0.057618
65	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	2356	22'3466'	-1.32158	0
C62	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	2346	22'344'	-1.01524	0.126408
C35	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	33'4	233'4'6/33'44'	-3.5252	0.223498
104	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'466'	22'355'6	-1.06303	0.054185
C44	8.74595	0.07589	0.10913	0.18271	-8.67007	-8.63683	-8.56324	-8.62338	0.05467	22'35'	2'3455'/22'33'56'/ 22'345'6/22'34'56	-0.26295	0.030016
C37,42	1.24749	0.02972	0.03335	0.05308	-1.21776	-1.21414	-1.19440	-1.20877	0.01257	344' 22'34'	22'344'6/22'34'5'6	-1.46123	0
72	0.01834	0.01415	0.00000	0.01151	-0.00419	-0.01834	-0.00683	-0.00978	0.00752	23'55'	233'45	-0.87141	0.06882
68	0.00000	0.03981	0.00000	0.06692	0.03981	0.00000	0.06692	0.03558	0.03366	23'45'	22'44'55'	-0.31008	0.037583
C41,64,71	2.83031	0.18397	0.36927	0.22664	-2.64634	-2.46104	-2.60367	-2.57035	0.09704	22'34 234'6 23'4'6	22'33'46'/233'44'	-0.36764	0.02866
C103	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'45'6			
57	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	233'5			

40	1.58257	0.02047	0.18955	0.05659	-1.56210	-1.39302	-1.52598	-1.49370	0.08904	22'33'			
C67,100	0.11284	0.01269	0.02269	0.02865	-0.10016	-0.09015	-0.08419	-0.09150	0.00807	23'45 22'44'6			
C63	0.00000	0.89707	0.67540	1.05596	0.89707	0.67540	1.05596	0.87615	0.19114	234'5			
C74,94	3.28613	0.06656	0.09427	0.33637	-3.21958	-3.19186	-2.94976	-3.12040	0.14843	244'5 22'356'			
61	0.00000	0.00985	0.01853	0.01115	0.00985	0.01853	0.01115	0.01318	0.00468	2345			
C70	5.13406	0.05279	0.06121	0.33970	-5.08127	-5.07284	-4.79435	-4.98282	0.16327	23'4'5			
98	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'346			
C102	0.00000	0.00000	0.00000	0.19614	0.00000	0.00000	0.19614	0.06538	0.11324	22'456'			
66	10.08022	0.00000	0.00000	0.00000	-10.08022	-10.08022	-10.08022	-10.08022	0.00000	23'44'			
93, 95	4.54984	0.12955	0.34931	0.37809	-4.42029	-4.20052	-4.17175	-4.26419	0.13595	22'356 22'35'6			
91	1.04216	0.03937	0.10525	0.13814	-1.00279	-0.93692	-0.90402	-0.94791	0.05030	22'34'6			
C55	0.03558	0.00000	0.00000	0.00000	-0.03558	-0.03558	-0.03558	-0.03558	0.00000	233'4			
C56,C60	6.32355	0.10937	0.13644	0.41433	-6.21418	-6.18712	-5.90922	-6.10351	0.16880	233'4' 2344'			
101	0.00000	0.07819	0.13140	0.37656	0.07819	0.13140	0.37656	0.19539	0.15914	22'455'			
C90	2.13301	0.00000	0.00000	0.00000	-2.13301	-2.13301	-2.13301	-2.13301	0.00000	22'34'5			
113	0.00000	0.00000	0.00000	0.02252	0.00000	0.00000	0.02252	0.00751	0.01300	233'5'6			
99	1.28974	0.00000	0.00000	0.15055	-1.28974	-1.28974	-1.13919	-1.23955	0.08692	22'44' 5			
79	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	33'45'			
112	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	233'56			
C78,83	0.17542	0.04739	0.04044	0.17530	-0.12803	-0.13498	-0.00012	-0.08771	0.07593	33'45 22'33'5			
C97	0.96354	0.16376	0.14350	0.25188	-0.79978	-0.82003	-0.71166	-0.77716	0.05762	22'3'45			
86	0.04803	0.00000	0.00000	0.02092	-0.04803	-0.04803	-0.02711	-0.04106	0.01208	22'345			
C81,87	0.00000	0.00000	0.07985	0.07513	0.00000	0.07985	0.07513	0.05166	0.04480	344'5 22'345'			
115	0.00000	0.05613	0.01672	0.09267	0.05613	0.01672	0.09267	0.05517	0.03798	2344'6			
145	1.32158	0.00000	0.00000	0.00000	-1.32158	-1.32158	-1.32158	-1.32158	0.00000	22'3466'			
120	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	23'455'			
85	1.15318	0.16559	0.00000	0.24824	-0.98760	-1.15318	-0.90494	-1.01524	0.12641	22'344'			
C136	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'66'			
C110,77	3.78192	0.17555	0.08515	0.50947	-3.60637	-3.69677	-3.27246	-3.52520	0.22350	233'4'6 33'44'			
C151	1.12764	0.03901	0.02798	0.12686	-1.08863	-1.09966	-1.00078	-1.06303	0.05419	22'355'6			
C124,135,144, 147	0.29160	0.02610	0.00000	0.05987	-0.26550	-0.29160	-0.23173	-0.26295	0.03002	2'3455' 22'33'56' 22'345'6 22'34'56			

C107,108	0.00000	0.01203	0.00000	0.02627	0.01203	0.00000	0.02627	0.01277	0.01315	233'4'5 233'45'			
139, 149	1.46123	0.00000	0.00000	0.00000	-1.46123	-1.46123	-1.46123	-1.46123	0.00000	22'344'6 22'34'5'6			
106	0.96619	0.03820	0.07474	0.17139	-0.92798	-0.89145	-0.79480	-0.87141	0.06882	233'45			
133	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'55'			
C134	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'56			
C114,131	0.00000	0.00000	0.00000	0.08652	0.00000	0.00000	0.08652	0.02884	0.04995	2344'5 22'33'46			
C165	0.00744	0.00000	0.00000	0.00000	-0.00744	-0.00744	-0.00744	-0.00744	0.00000	233'55'6			
C146	0.12178	0.01493	0.00000	0.04144	-0.10686	-0.12178	-0.08034	-0.10299	0.02099	22'34'55'			
C161	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	233'45'6			
184	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'344'66'			
153	0.37155	0.02256	0.06428	0.09756	-0.34899	-0.30727	-0.27399	-0.31008	0.03758	22'44'55'			
168	0.00000	0.00000	0.00000	0.00524	0.00000	0.00000	0.00524	0.00175	0.00303	23'44'5'6			
127	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	33'455'			
132, 105	0.39750	0.01407	0.01257	0.06294	-0.38344	-0.38493	-0.33456	-0.36764	0.02866	22'33'46' 233'44'			
179	0.00000	0.01137	0.01475	0.03221	0.01137	0.01475	0.03221	0.01944	0.01119	22'33'566'			
141	0.00000	0.00000	0.06071	0.05261	0.00000	0.06071	0.05261	0.03777	0.03296	22'3455'			
137, 176, 130	0.01213	0.00000	0.02042	0.01915	-0.01213	0.00829	0.00702	0.00106	0.01144	22'344'5 22'33'466' 22'33'45'			
164	0.00000	0.00993	0.00000	0.00000	0.00993	0.00000	0.00000	0.00331	0.00573	233'4'5'6			
163, 138	0.12316	0.01295	0.01528	0.03328	-0.11020	-0.10787	-0.08988	-0.10265	0.01112	233'4'56 22344'5'			
C158	0.00000	0.03360	0.03408	0.00000	0.03360	0.03408	0.00000	0.02256	0.01954	233'44'6			
160	0.02474	0.00000	0.00629	0.01991	-0.02474	-0.01845	-0.00484	-0.01601	0.01018	234'456			
186	0.00000	0.00807	0.00722	0.01501	0.00807	0.00722	0.01501	0.01010	0.00427	22'34566'			
C126,129,178	0.03336	0.01433	0.03896	0.03773	-0.01903	0.00560	0.00437	-0.00302	0.01388	33'44'5 22'33'45 22'33'55'6			
C175	0.00000	0.00000	0.01452	0.00470	0.00000	0.01452	0.00470	0.00641	0.00741	22'33'45'6			
159	0.00000	0.00000	0.01101	0.00000	0.00000	0.01101	0.00000	0.00367	0.00636	233'455'			
186, 182	0.00000	0.01210	0.00000	0.05533	0.01210	0.00000	0.05533	0.02247	0.02909	22'34566' 22'344'56'			
C183	0.00000	0.00000	0.00000	0.09960	0.00000	0.00000	0.09960	0.03320	0.05750	22'344'5'6			
C167	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	23'44'55'			
C128	0.00000	0.00000	0.03085	0.05791	0.00000	0.03085	0.05791	0.02959	0.02897	22'33'44'			
185	0.00000	0.00000	0.03927	0.00000	0.00000	0.03927	0.00000	0.01309	0.02267	22'3455'6			
C174	0.00000	0.01601	0.02292	0.02068	0.01601	0.02292	0.02068	0.01987	0.00352	22'33'456'			

181	0.01536	0.00000	0.00000	0.00000	-0.01536	-0.01536	-0.01536	-0.01536	0.00000	22'344'56			
C177	0.03358	0.00000	0.03230	0.00000	-0.03358	-0.00128	-0.03358	-0.02281	0.01865	22'33'4'56			
202, 171	0.00000	0.00000	0.00000	0.00410	0.00000	0.00000	0.00410	0.00137	0.00237	22'33'55'66' 22'33'44'6			
	0.00000	0.00000	0.00000	0.01897	0.00000	0.00000	0.01897	0.00632	0.01095	233'44'5			
C173	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'456			
C197	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'44'66'			
C192	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	233'455'6			
C180	0.02209	0.00000	0.00000	0.01000	-0.02209	-0.02209	-0.01209	-0.01876	0.00578	22'344'55'			
C193	0.00000	0.00000	0.10306	0.00000	0.00000	0.10306	0.00000	0.03435	0.05950	233'4'55'6			
C191	0.00000	0.00000	0.02697	0.00000	0.00000	0.02697	0.00000	0.00899	0.01557	233'44'5'6			
C199	0.00000	0.04239	0.00000	0.00000	0.04239	0.00000	0.00000	0.01413	0.02448	22'33'4566'			
169	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	33'44'55'			
C170,190	0.01886	0.01099	0.00000	0.01295	-0.00787	-0.01886	-0.00591	-0.01088	0.00698	22'33'44'5 233'44'56			
198	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'455'6			
201	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	22'33'45'66'			
C196,203	0.00000	0.00883	0.07829	0.01209	0.00883	0.07829	0.01209	0.03307	0.03920	22'344'55'6 22'33'44'56'			
C195,208	0.00000	0.00000	0.04179	0.00817	0.00000	0.04179	0.00817	0.01666	0.02215	22'33'455'66' 22'33'44'56			
C207	0.00000	0.00000	0.00000	0.02780	0.00000	0.00000	0.02780	0.00927	0.01605	22'33'44'566'			
C194	0.00000	0.04246	0.12056	0.01638	0.04246	0.12056	0.01638	0.05980	0.05421	22'33'44'55'			
C205	0.00000	0.06210	0.09450	0.01440	0.06210	0.09450	0.01440	0.05700	0.04029	233'44'55'6'			
C206	0.00000	0.00000	0.10787	0.24727	0.00000	0.10787	0.24727	0.11838	0.12397	22'33'44'55'6			
C209	0.01633	0.15401	0.19120	0.11413	0.13769	0.17487	0.09781	0.13679	0.03854	22'33'44'55'66'			

D0							
RetTime	Amount	Name	Configuration	lecular wei	Rel mol pre:	Mol%	ln %
[min]	[ug/L]				(mass%/mo	rel mol pres/total mol pres	
33.356		C1	2	188.631	0.00	0	0
37.091		3	4	188.631	0.00	0	0
39.375	3.21E-01	C4,10	22' 26	223.084	0.14	0.005373391	0.537339
41.686	7.48E-02	C7,9	24 25	223.084	0.03	0.001250651	0.125065
42.883	6.64E-02	C6	23'	223.084	0.03	0.001110262	0.111026
43.463		C5,8	23 24'	223.084	0.00	0	0
44.806		C14	35	223.084	0.00	0	0
45.674	5.21E-01	C19	22'6	257.537	0.20	0.007551853	0.755185
46.765		C11	33'	223.084	0.00	0	0
47.457		12, 13	34, 34'	223.084	0.00	0	0
47.824	6.94E-01	C18	22'5	257.537	0.27	0.010049399	1.00494
48.019	2.90E-01	C15,17	44' 22'4	240.31	0.12	0.004500449	0.450045
48.87	3.39E-02	C24,27	236 23'6	257.537	0.01	0.000490833	0.049083
49.703	6.06E-01	C16,32	22'3 24'6	257.537	0.24	0.008775472	0.877547
50.555	28.28969	23	235	257.537	10.98	0.409901811	40.99018
50.819		29	245	257.537	0.00	0	0
51.082		C54	22'66'	291.99	0.00	0	0
51.356	1.90E-01	C26	23'5	257.537	0.07	0.002754979	0.275498
51.618	8.15E-02	C25	23'4	257.537	0.03	0.00118156	0.118156
52.141	1.16333	C31	24'5	257.537	0.45	0.016856002	1.6856
52.185		50	22'46	291.99	0.00	0	0
52.28	1.71663	28	244'	257.537	0.67	0.02487301	2.487301
53.276	2.18E-01	C21,33	234 2'34	257.537	0.08	0.003151598	0.31516
53.405	1.50E-01	53	22'56'	291.99	0.05	0.001921912	0.192191
53.853	3.88E-02	C51	22'46'	291.99	0.01	0.000495693	0.049569
54.014	4.13E-01		33'5	257.537	0.16	0.005979604	0.59796
54.579	4.36E-01		22'36	291.99	0.15	0.005577581	0.557758
55.159			34'5	257.537	0.00	0	0
55.364	2.50E-01	C46	22'36'	291.99	0.09	0.003191494	0.319149
55.572	9.40E-01	52,	22'55'	291.99	0.32	0.012018755	1.201875
55.583		C73	23'5'6	291.99	0.00	0	0
55.984	7.43E-01	22	234'	257.537	0.29	0.010771335	1.077133
56.075		C49	22'45'	291.99	0.00	0	0
56.2		38	345	257.537	0.00	0	0
56.244	2.82E-01	C47,75	22'44' 244'6	291.99	0.10	0.003599897	0.35999
56.402	8.72E-01	C48	22'45	291.99	0.30	0.011139647	1.113965
56.629		65	2356	291.99	0.00	0	0
56.684		C62	2346	291.99	0.00	0	0
57.079		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.598	4.01622	C44	22'35'	291.99	1.38	0.051326399	5.13264
57.917	5.93E-01	C37,42	344' 22'34'	291.99	0.20	0.007575788	0.757579
58.195	1.05E-02	72	23'55'	291.99	0.00	0.000133872	0.013387
58.657		68	23'45'	291.99	0.00	0	0
58.719	1.3687	C41,64,71	22'34 234'6 23'	291.99	0.47	0.017491682	1.749168
59.417		C103	22'45'6	326.44	0.00	0	0
59.46		57	233'5	291.99	0.00	0	0
59.585	7.30E-01	40	22'33'	291.99	0.25	0.009328778	0.932878
59.915	6.10E-02	C67,100	23'45 22'44'6	309.22	0.02	0.000735835	0.073583

60.409	3.26E-01	C63	234'5	291.99	0.11	0.00416447	0.416447
60.768	1.55752	C74,94	244'5 22'3	326.44	0.48	0.017804162	1.780416
60.921		61	2345	291.99	0.00	0	0
61.134	4.25394	C70	23'4'5	291.99	1.46	0.054364408	5.436441
61.283		98	22'346	326.44	0.00	0	0
61.4		C102	22'456'	326.44	0.00	0	0
61.481	6.23564	66	23'44'	291.99	2.14	0.079690094	7.969009
61.644	3.96807	93, 95	22'356 22'35'6	326.44	1.22	0.045359393	4.535939
62.093		91	22'34'6	326.44	0.00	0	0
62.215	1.43E-01	C55	233'4	291.99	0.05	0.001829259	0.182926
63.102	3.43597	C56,C60	233'4' 2344'	291.99	1.18	0.043910933	4.391093
63.578		101	22'455'	326.44	0.00	0	0
63.632		C90	22'34'5	326.44	0.00	0	0
63.85	9.04E-02	113	233'5'6	326.44	0.03	0.001033011	0.103301
64.033		99	22'44' 5	326.44	0.00	0	0
64.094	8.92E-01	79	33'45'	291.99	0.31	0.011394961	1.139496
64.935	5.96E-02	112	233'56	326.44	0.02	0.000680864	0.068086
65.184	2.38E-01	C78,83	33'45 22'33'5	326.44	0.07	0.002723916	0.272392
65.65	7.90E-01	C97	22'3'45	326.44	0.24	0.009033082	0.903308
65.797	5.60E-02	86	22'345	326.44	0.02	0.000639656	0.063966
66.046		C81,87	344'5 22'345'	326.44	0.00	0	0
66.08		115	2344'6	326.44	0.00	0	0
66.115	1.21768	145	22'3466'	360.88	0.34	0.012591041	1.259104
66.19		120	23'455'	326.44	0.00	0	0
66.495	9.19E-01	85	22'344'	326.44	0.28	0.010503669	1.050367
66.8		C136	22'33'66'	360.88	0.00	0	0
67.011	2.40403	C110,77	233'4'6 33'44'	309.22	0.78	0.029011529	2.901153
68.098	6.82E-01	C151	22'355'6	326.44	0.21	0.007801289	0.780129
68.485	1.92E-01	C124,135	2'3455' 22'33'56	360.88	0.05	0.001981614	0.198161
68.703		C107,108	233'4'5 233'45	326.44	0.00	0	0
68.794	7.33E-01	139, 149	22'344'6 22'3	360.88	0.20	0.007575894	0.757589
69.228	6.16E-01	106	233'45	326.44	0.19	0.007037383	0.703738
69.812		133	22'33'55'	360.88	0.00	0	0
70.1		C134	22'33'56	360.88	0.00	0	0
70.271	3.12E-01	C114,131	2344'5 22'33'46	360.88	0.09	0.003226739	0.322674
70.44		C165	233'55'6	360.88	0.00	0	0
70.673	8.33E-02	C146	22'34'55'	360.88	0.02	0.000860855	0.086085
70.725		C161	233'45'6	360.88	0.00	0	0
70.881		184	22'344'66'	395.33	0.00	0	0
71.19	2.70E-01	153	22'44'55'	360.88	0.07	0.002795966	0.279597
71.35		168	23'44'5'6	360.88	0.00	0	0
71.446		127	33'455'	326.44	0.00	0	0
71.586		132, 105	22'33'46'	360.88	0.00	0	0
71.663	3.90E-01	179	22'33'566'	395.33	0.10	0.003681965	0.368196
72.677		141	22'3455'	360.88	0.00	0	0
73.235	1.97E-02	137, 176,	22'344'5 22'3	360.88	0.01	0.000203335	0.020333
73.525		164	233'4'5'6	360.88	0.00	0	0
73.703	9.58E-02	163, 138	233'4'56 2234	360.88	0.03	0.000990969	0.099097
73.83		C158	233'44'6	360.88	0.00	0	0
73.92		160	234'456	360.88	0.00	0	0
74.145		186	22'34566'	395.33	0.00	0	0
74.478		C126,129	33'44'5 22'33'45	360.88	0.00	0	0

74.85		C175	22'33'45'6	395.33	0.00	0	0
75.05		159	233'455'	360.88	0.00	0	0
75.151		186, 182	22'34566' 22'34	395.33	0.00	0	0
75.59		C183	22'344'5'6	395.33	0.00	0	0
76.082		C167	23'44'55'	360.88	0.00	0	0
76.177		C128	22'33'44'	360.88	0.00	0	0
76.489		185	22'3455'6	395.33	0.00	0	0
77.175		C174	22'33'456'	395.33	0.00	0	0
77.257		181	22'344'56	395.33	0.00	0	0
77.711		C177	22'33'4'56	395.33	0.00	0	0
77.827		202, 171	22'33'55'66' 2	429.78	0.00	0	0
78.117			233'44'5	360.88	0.00	0	0
78.518		C173	22'33'456	395.33	0.00	0	0
78.857		C197	22'33'44'66'	395.33	0.00	0	0
78.92		C192	233'455'6	395.33	0.00	0	0
79.501		C180	22'344'55'	395.33	0.00	0	0
79.77		C193	233'4'55'6	395.33	0.00	0	0
80.013		C191	233'44'5'6	395.33	0.00	0	0
80.646		C199	22'33'4566'	429.78	0.00	0	0
81.105		169	33'44'55'	360.88	0.00	0	0
82.096		C170,190	22'33'44'5 233'44	395.33	0.00	0	0
82.331		198	22'33'455'6	429.78	0.00	0	0
82.712		201	22'33'45'66'	429.78	0.00	0	0
83.26		C196,203	22'344'55'6 22'	429.78	0.00	0	0
85.732		C195,208	22'33'455'66'	464.23	0.00	0	0
86.272		C207	22'33'44'566'	464.23	0.00	0	0
87.263		C194	22'33'44'55'	429.78	0.00	0	0
87.737		C205	233'44'55'6'	429.78	0.00	0	0
90.39		C206	22'33'44'55'6	464.23	0.00	0	0
92.518		C209	22'33'44'55'66'	498.68	0.00	0	0
Total	75.150131				**26.80**	**1.00**	**100.00**

D28

RetTime [min]	Amount [ug/L]	Name	Configuration	lecular wei	Rel mol pre: (mass%/mo	Mol% rel mol pres/total mol pres	In %
33.357		C1	2	188.631	0.00	0	0
37.056	1.56955	3	4	188.631	0.83	0.022423416	2.242342
39.318		C4,10	22' 26	223.084	0.00	0	0
41.734	6.30E-02	C7,9	24 25	223.084	0.03	0.000760515	0.076051
42.736	8.16E-02	C6	23'	223.084	0.04	0.000985276	0.098528
43.516	7.46E-01	C5,8	23 24'	223.084	0.33	0.00901624	0.901624
44.784	2.36E-02	C14	35	223.084	0.01	0.000284876	0.028488
45.675	3.67E-01	C19	22'6	257.537	0.14	0.003845342	0.384534
47.11	3.67284	C11	33'	223.084	1.65	0.04436835	4.436835
47.317		12, 13	34, 34'	223.084	0.00	0	0
47.82	5.91E-01	C18	22'5	257.537	0.23	0.006186831	0.618683
48.174	8.36E-01	C15,17	44' 22'4	240.31	0.35	0.009375513	0.937551
48.861	4.43E-02	C24,27	236 23'6	257.537	0.02	0.000463978	0.046398
49.696	4.76E-01	C16,32	22'3 24'6	257.537	0.18	0.004980619	0.498062
50.553	10.12806	23	235	257.537	3.93	0.105980587	10.59806
50.87		29	245	257.537	0.00	0	0
51.099	2.34E-02	C54	22'66'	291.99	0.01	0.000216284	0.021628
51.356	1.84E-01	C26	23'5	257.537	0.07	0.00192795	0.192795
51.613	7.79E-02	C25	23'4	257.537	0.03	0.000815467	0.081547
52.138	1.17804	C31	24'5	257.537	0.46	0.012327077	1.232708
52.185		50	22'46	291.99	0.00	0	0
52.278	1.75094	28	244'	257.537	0.68	0.018321934	1.832193
53.272	2.09E-01	C21,33	234 2'34	257.537	0.08	0.002190064	0.219006
53.401	1.45E-01	53	22'56'	291.99	0.05	0.001336836	0.133684
53.848	5.11E-02	C51	22'46'	291.99	0.02	0.000471962	0.047196
54.01	4.27E-01		33'5	257.537	0.17	0.004464667	0.446467
54.577	4.34E-01		22'36	291.99	0.15	0.004004122	0.400412
54.988			34'5	257.537	0.00	0	0
55.361	2.48E-01	C46	22'36'	291.99	0.09	0.002291309	0.229131
55.568	1.09884	52,	22'55'	291.99	0.38	0.010141593	1.014159
55.607		C73	23'5'6	291.99	0.00	0	0
55.979	9.00E-01	22	234'	257.537	0.35	0.009414982	0.941498
56.075		C49	22'45'	291.99	0.00	0	0
56.2		38	345	257.537	0.00	0	0
56.24	3.67E-01	C47,75	22'44' 244'6	291.99	0.13	0.003384121	0.338412
56.395	1.03435	C48	22'45	291.99	0.35	0.009546391	0.954639
56.629		65	2356	291.99	0.00	0	0
56.871		C62	2346	291.99	0.00	0	0
57.124		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.593	4.55006	C44	22'35'	291.99	1.56	0.041994154	4.199415
57.912	6.99E-01	C37,42	344' 22'34'	291.99	0.24	0.00645258	0.645258
58.191	1.58E-02	72	23'55'	291.99	0.01	0.000145416	0.014542
58.557		68	23'45'	291.99	0.00	0	0
58.714	1.63075	C41,64,71	22'34 234'6 23'4'	291.99	0.56	0.015050783	1.505078
59.3	5.70E-02	C103	22'45'6	326.44	0.02	0.000470246	0.047025
59.46		57	233'5	291.99	0.00	0	0
59.581	7.97E-01	40	22'33'	291.99	0.27	0.007357491	0.735749
59.91	8.83E-02	C67,100	23'45 22'44'6	309.22	0.03	0.000769683	0.076968

60.326	2.30898	C63	234'5	291.99	0.79	0.021310414	2.131041
60.766	2.38735	C74,94	244'5 22'35	326.44	0.73	0.019708447	1.970845
60.916	27.75783	61	2345	291.99	9.51	0.256187079	25.61871
61.128	5.48633	C70	23'4'5	291.99	1.88	0.050635329	5.063533
61.312		98	22'346	326.44	0.00	0	0
61.439		C102	22'456'	326.44	0.00	0	0
61.475	7.33755	66	23'44'	291.99	2.51	0.06772091	6.772091
61.639	3.42858	93, 95	22'356 22'35'6	326.44	1.05	0.028304182	2.830418
62.209	1.06066	91	22'34'6	326.44	0.32	0.008756136	0.875614
62.303		C55	233'4	291.99	0.00	0	0
63.096	4.79464	C56,C60	233'4' 2344'	291.99	1.64	0.044251471	4.425147
63.6		101	22'455'	326.44	0.00	0	0
63.617	3.53522	C90	22'34'5	326.44	1.08	0.029184534	2.918453
63.785		113	233'5'6	326.44	0.00	0	0
64.089	1.53772	99	22'44' 5	326.44	0.47	0.012694441	1.269444
64.158		79	33'45'	291.99	0.00	0	0
64.931	4.56E-02	112	233'56	326.44	0.01	0.000376673	0.037667
65.176	2.79E-01	C78,83	33'45 22'33'5	326.44	0.09	0.002300894	0.230089
65.642	1.08344	C97	22'3'45	326.44	0.33	0.008944193	0.894419
65.791	6.91E-02	86	22'345	326.44	0.02	0.000570186	0.057019
65.952		C81,87	344'5 22'345'	326.44	0.00	0	0
66.107	5.63E-01	115	2344'6	326.44	0.17	0.004647267	0.464727
66.14		145	22'3466'	360.88	0.00	0	0
66.19		120	23'455'	326.44	0.00	0	0
66.495	1.33519	85	22'344'	326.44	0.41	0.011022482	1.102248
66.8		C136	22'33'66'	360.88	0.00	0	0
67.002	3.59938	C110,77	233'4'6 33'44'	309.22	1.16	0.031369445	3.136944
68.094	1.0827	C151	22'355'6	326.44	0.33	0.008938084	0.893808
68.481	3.38E-01	C124,135,144,	2'3455' 22'33'56'	360.88	0.09	0.002523061	0.252306
68.703		C107,108	233'4'5 233'45'	326.44	0.00	0	0
68.784	7.73E-01	139, 149	22'344'6 22'34'	360.88	0.21	0.005769166	0.576917
69.221	1.04576	106	233'45	326.44	0.32	0.008633131	0.863313
69.855		133	22'33'55'	360.88	0.00	0	0
70.1		C134	22'33'56	360.88	0.00	0	0
70.264	5.75E-01	C114,131	2344'5 22'33'46	360.88	0.16	0.00429524	0.429524
70.524	1.02E-02	C165	233'55'6	360.88	0.00	7.62957E-05	0.00763
70.66	1.46E-01	C146	22'34'55'	360.88	0.04	0.001093097	0.10931
70.725		C161	233'45'6	360.88	0.00	0	0
70.981		184	22'344'66'	395.33	0.00	0	0
71.187	4.77E-01	153	22'44'55'	360.88	0.13	0.003562481	0.356248
71.35		168	23'44'5'6	360.88	0.00	0	0
71.375		127	33'455'	326.44	0.00	0	0
71.655	4.90E-01	132, 105	22'33'46'	360.88	0.14	0.003662524	0.366252
71.845		179	22'33'566'	395.33	0.00	0	0
72.665	4.29E-02	141	22'3455'	360.88	0.01	0.000320081	0.032008
73.23	1.92E-02	137, 176, 130	22'344'5 22'33'	360.88	0.01	0.000143217	0.014322
73.525		164	233'4'5'6	360.88	0.00	0	0
73.697	1.65E-01	163, 138	233'4'56 22344	360.88	0.05	0.001232881	0.123288
73.831		C158	233'44'6	360.88	0.00	0	0
73.917		160	234'456	360.88	0.00	0	0
74.145		186	22'34566'	395.33	0.00	0	0
74.468		C126,129,178	33'44'5 22'33'45	360.88	0.00	0	0

74.732	C175	22'33'45'6	395.33	0.00	0	0
75.05	159	233'455'	360.88	0.00	0	0
75.142	186, 182	22'34566' 22'344	395.33	0.00	0	0
75.582	C183	22'344'5'6	395.33	0.00	0	0
76.082	C167	23'44'55'	360.88	0.00	0	0
76.167	C128	22'33'44'	360.88	0.00	0	0
76.489	185	22'3455'6	395.33	0.00	0	0
77.175	C174	22'33'456'	395.33	0.00	0	0
77.256	181	22'344'56	395.33	0.00	0	0
77.71	C177	22'33'4'56	395.33	0.00	0	0
77.9	202, 171	22'33'55'66' 22'	429.78	0.00	0	0
78.102		233'44'5	360.88	0.00	0	0
78.518	C173	22'33'456	395.33	0.00	0	0
78.857	C197	22'33'44'66'	395.33	0.00	0	0
78.92	C192	233'455'6	395.33	0.00	0	0
79.495	C180	22'344'55'	395.33	0.00	0	0
79.755	C193	233'4'55'6	395.33	0.00	0	0
80.013	C191	233'44'5'6	395.33	0.00	0	0
80.646	C199	22'33'4566'	429.78	0.00	0	0
81.105	169	33'44'55'	360.88	0.00	0	0
82.088	C170,190	22'33'44'5 233'44'5	395.33	0.00	0	0
82.343	198	22'33'455'6	429.78	0.00	0	0
82.79	201	22'33'45'66'	429.78	0.00	0	0
83.253	C196,203	22'344'55'6 22'33	429.78	0.00	0	0
85.732	C195,208	22'33'455'66'	464.23	0.00	0	0
86.327	C207	22'33'44'566'	464.23	0.00	0	0
87.348	C194	22'33'44'55'	429.78	0.00	0	0
87.8	C205	233'44'55'6'	429.78	0.00	0	0
90.202	C206	22'33'44'55'6	464.23	0.00	0	0
92.516	C209	22'33'44'55'66'	498.68	0.00	0	0
Total 106.3409796				**37.11**	**1.00**	**100.00**

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RetTime	Amount	Name	Configuration	lecular wei	Rel mol pre	Mol%	In %
[min]	[ug/L]				(mass%/mo	rel mol pres/total mol pres	
33.342		C1	2	188.631	0.00	0	0
37.065	1.92194	3	4	188.631	1.02	0.040205555	4.020555
39.413	2.77811	C4,10	22' 26	223.084	1.25	0.049140584	4.914058
41.817		C7,9	24 25	223.084	0.00	0	0
42.946	5.00E-01	C6	23'	223.084	0.22	0.008850173	0.885017
43.46	1.66	C5,8	23 24'	223.084	0.74	0.029362901	2.93629
44.759	9.52E-02	C14	35	223.084	0.04	0.001683106	0.168311
45.724	1.48587	C19	22'6	257.537	0.58	0.022766721	2.276672
46.793		C11	33'	223.084	0.00	0	0
47.457		12, 13	34, 34'	223.084	0.00	0	0
47.679	5.46E-02	C18	22'5	257.537	0.02	0.000836857	0.083686
48.018		C15,17	44' 22'4	240.31	0.00	0	0
48.732	5.40E-02	C24,27	236 23'6	257.537	0.02	0.000827341	0.082734
49.671	1.05E-01	C16,32	22'3 24'6	257.537	0.04	0.001601731	0.160173
50.534	15.89017	23	235	257.537	6.17	0.243471546	24.34715
50.904		29	245	257.537	0.00	0	0
51.097	1.80E-02	C54	22'66'	291.99	0.01	0.000242964	0.024296
51.355		C26	23'5	257.537	0.00	0	0
51.616		C25	23'4	257.537	0.00	0	0
52.127	1.23E-01	C31	24'5	257.537	0.05	0.001885222	0.188522
52.185		50	22'46	291.99	0.00	0	0
52.275	2.05E-01	28	244'	257.537	0.08	0.003146403	0.31464
53.261	2.80E-02	C21,33	234 2'34	257.537	0.01	0.000428539	0.042854
53.389	1.68E-02	53	22'56'	291.99	0.01	0.000226732	0.022673
53.854	1.76E-02	C51	22'46'	291.99	0.01	0.000237407	0.023741
54.018	1.19E-01		33'5	257.537	0.05	0.001827687	0.182769
54.555	1.60E-01		22'36	291.99	0.05	0.00216402	0.216402
55.159			34'5	257.537	0.00	0	0
55.367	1.61E-01	C46	22'36'	291.99	0.06	0.002173034	0.217303
55.555	1.55E-01	52,	22'55'	291.99	0.05	0.002097503	0.20975
55.583		C73	23'5'6	291.99	0.00	0	0
55.964	1.47E-01	22	234'	257.537	0.06	0.002250471	0.225047
56.075		C49	22'45'	291.99	0.00	0	0
56.22	1.01E-01	38	345	257.537	0.04	0.001541132	0.154113
56.243		C47,75	22'44' 244'6	291.99	0.00	0	0
56.382	1.48E-01	C48	22'45	291.99	0.05	0.001997349	0.199735
56.664	6.13E-03	65	2356	291.99	0.00	8.28294E-05	0.008283
56.742		C62	2346	291.99	0.00	0	0
57.127		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.577	5.43E-01	C44	22'35'	291.99	0.19	0.007334903	0.73349
57.897	9.37E-02	C37,42	344' 22'34'	291.99	0.03	0.00126586	0.126586
58.193		72	23'55'	291.99	0.00	0	0
58.657		68	23'45'	291.99	0.00	0	0
58.7	1.98E-01	C41,64,7	22'34 234'6 23'4'	291.99	0.07	0.002670398	0.26704
59.417		C103	22'45'6	326.44	0.00	0	0
59.46		57	233'5	291.99	0.00	0	0
59.563	9.07E-02	40	22'33'	291.99	0.03	0.001225849	0.122585

59.888	3.46E-02	C67,100	23'45	22'44'6	309.22	0.01	0.000441726	0.044173
60.302	3.1279	C63	234'5		291.99	1.07	0.042271162	4.227116
60.772		C74,94	244'5	22'35	326.44	0.00	0	0
60.895	28.91904	61	2345		291.99	9.90	0.390818574	39.08186
61.111	1.94997	C70	23'4'5		291.99	0.67	0.026352344	2.635234
61.283		98	22'346		326.44	0.00	0	0
61.4		C102	22'456'		326.44	0.00	0	0
61.461	1.35227	66	23'44'		291.99	0.46	0.018274889	1.827489
61.618	6.95E-01	93, 95	22'356	22'35'6	326.44	0.21	0.008401999	0.8402
62.192	2.15E-01	91	22'34'6		326.44	0.07	0.002598143	0.259814
62.212		C55	233'4		291.99	0.00	0	0
63.084	8.53E-01	C56,C60	233'4'	2344'	291.99	0.29	0.011526558	1.152656
63.595	8.99E-01	101	22'455'		326.44	0.28	0.01086669	1.086669
63.632		C90	22'34'5		326.44	0.00	0	0
63.832	2.11E-02	113	233'5'6		326.44	0.01	0.000255535	0.025554
64.033		99	22'44' 5		326.44	0.00	0	0
64.071	4.03E-01	79	33'45'		291.99	0.14	0.005448289	0.544829
64.872		112	233'56		326.44	0.00	0	0
65.159	1.33E-01	C78,83	33'45	22'33'5	326.44	0.04	0.001601798	0.16018
65.625	3.80E-01	C97	22'3'45		326.44	0.12	0.004596055	0.459605
65.768	2.61E-02	86	22'345		326.44	0.01	0.000314904	0.03149
65.971	6.54E-02	C81,87	344'5	22'345'	326.44	0.02	0.0007906	0.07906
66.09	1.65E-01	115	2344'6		326.44	0.05	0.001989837	0.198984
66.14		145	22'3466'		360.88	0.00	0	0
66.19		120	23'455'		326.44	0.00	0	0
66.473	3.73E-01	85	22'344'		326.44	0.11	0.004503533	0.450353
66.8		C136	22'33'66'		360.88	0.00	0	0
66.985	9.23E-01	C110,77	233'4'6	33'44'	309.22	0.30	0.01178459	1.178459
68.072	2.98E-01	C151	22'355'6		326.44	0.09	0.003599057	0.359906
68.457	1.06E-01	C124,135	2'3455'	22'33'56'	360.88	0.03	0.001162287	0.116229
68.657		C107,108	233'4'5	233'45'	326.44	0.00	0	0
68.765	7.06E-01	139, 149	22'344'6	22'34'	360.88	0.20	0.007717636	0.771764
69.202	3.38E-01	106	233'45		326.44	0.10	0.004088103	0.40881
69.698		133	22'33'55'		360.88	0.00	0	0
70.102		C134	22'33'56		360.88	0.00	0	0
70.252	3.05E-01	C114,131	2344'5	22'33'46	360.88	0.08	0.003330849	0.333085
70.44		C165	233'55'6		360.88	0.00	0	0
70.644	7.72E-02	C146	22'34'55'		360.88	0.02	0.000843881	0.084388
70.725		C161	233'45'6		360.88	0.00	0	0
70.805		184	22'344'66'		395.33	0.00	0	0
71.165	1.84E-01	153	22'44'55'		360.88	0.05	0.002009312	0.200931
71.35		168	23'44'5'6		360.88	0.00	0	0
71.375		127	33'455'		326.44	0.00	0	0
71.506		132, 105	22'33'46'		360.88	0.00	0	0
71.637	2.06E-01	179	22'33'566'		395.33	0.05	0.00205992	0.205992
72.677		141	22'3455'		360.88	0.00	0	0
73.213	9.19E-03	137, 176,	22'344'5	22'33'	360.88	0.00	0.000100481	0.010048
73.525		164	233'4'5'6		360.88	0.00	0	0
73.676	6.46E-02	163, 138	233'4'56	22344	360.88	0.02	0.000706428	0.070643
73.83		C158	233'44'6		360.88	0.00	0	0
73.889		160	234'456		360.88	0.00	0	0
74.145		186	22'34566'		395.33	0.00	0	0

74.447		C126,129	33'44'5 22'33'45	360.88	0.00	0	0
74.85		C175	22'33'45'6	395.33	0.00	0	0
75.05		159	233'455'	360.88	0.00	0	0
75.124		186, 182	22'34566' 22'344	395.33	0.00	0	0
75.771		C183	22'344'5'6	395.33	0.00	0	0
76.144		C167	23'44'55'	360.88	0.00	0	0
76.172		C128	22'33'44'	360.88	0.00	0	0
76.376		185	22'3455'6	395.33	0.00	0	0
77.175		C174	22'33'456'	395.33	0.00	0	0
77.228		181	22'344'56	395.33	0.00	0	0
77.682		C177	22'33'4'56	395.33	0.00	0	0
77.9		202, 171	22'33'55'66' 22'	429.78	0.00	0	0
78.091			233'44'5	360.88	0.00	0	0
78.518		C173	22'33'456	395.33	0.00	0	0
78.857		C197	22'33'44'66'	395.33	0.00	0	0
78.92		C192	233'455'6	395.33	0.00	0	0
79.353		C180	22'344'55'	395.33	0.00	0	0
79.659		C193	233'4'55'6	395.33	0.00	0	0
80.013		C191	233'44'5'6	395.33	0.00	0	0
80.697		C199	22'33'4566'	429.78	0.00	0	0
81.105		169	33'44'55'	360.88	0.00	0	0
82.054		C170,190	22'33'44'5 233'44'5	395.33	0.00	0	0
82.434		198	22'33'455'6	429.78	0.00	0	0
82.765		201	22'33'45'66'	429.78	0.00	0	0
83.235		C196,203	22'344'55'6 22'33	429.78	0.00	0	0
85.732		C195,208	22'33'455'66'	464.23	0.00	0	0
86.272		C207	22'33'44'566'	464.23	0.00	0	0
87.263		C194	22'33'44'55'	429.78	0.00	0	0
87.737		C205	233'44'55'6'	429.78	0.00	0	0
90.39		C206	22'33'44'55'6	464.23	0.00	0	0
92.487		C209	22'33'44'55'66'	498.68	0.00	0	0
Total	69.70348				**25.34**	**1.00**	**100.00**

D135A

RetTime [min]	Amount [ug/L]	Name	Configuration	Molecular	Rel mol pres (mass%/mo	Mol% rel mol pres/total mol pres	In %
33.325		C1	2	188.631	0.00	0	0
37.037		3	4	188.631	0.00	0	0
39.318		C4,10	22' 26	223.084	0.00	0	0
41.762		C7,9	24 25	223.084	0.00	0	0
42.881		C6	23'	223.084	0.00	0	0
43.539		C5,8	23 24'	223.084	0.00	0	0
44.806		C14	35	223.084	0.00	0	0
45.661		C19	22'6	257.537	0.00	0	0
47.081	6.97E-01	C11	33'	223.084	0.31	0.069459647	6.945965
47.317		12, 13	34, 34'	223.084	0.00	0	0
47.775		C18	22'5	257.537	0.00	0	0
48.139	5.22E-01	C15,17	44' 22'4	240.31	0.22	0.048283418	4.828342
48.807		C24,27	236 23'6	257.537	0.00	0	0
49.704	3.56E-02	C16,32	22'3 24'6	257.537	0.01	0.003070699	0.30707
50.523	1.16251	23	235	257.537	0.45	0.100404983	10.0405
50.87		29	245	257.537	0.00	0	0
51.082		C54	22'66'	291.99	0.00	0	0
51.323		C26	23'5	257.537	0.00	0	0
51.598	1.34E-02	C25	23'4	257.537	0.01	0.001156759	0.115676
52.119	4.25E-02	C31	24'5	257.537	0.02	0.003671889	0.367189
52.185		50	22'46	291.99	0.00	0	0
52.26	7.82E-02	28	244'	257.537	0.03	0.006755016	0.675502
53.252	7.63E-03	C21,33	234 2'34	257.537	0.00	0.000659389	0.065939
53.371	7.63E-03	53	22'56'	291.99	0.00	0.000581055	0.058106
53.812		C51	22'46'	291.99	0.00	0	0
54.012	2.19E-02		33'5	257.537	0.01	0.00188866	0.188866
54.546	2.47E-02		22'36	291.99	0.01	0.001878971	0.187897
54.99	9.42E-03		34'5	257.537	0.00	0.000813979	0.081398
55.334	2.90E-02	C46	22'36'	291.99	0.01	0.002211771	0.221177
55.539	6.89E-02	52,	22'55'	291.99	0.02	0.005245526	0.524553
55.607		C73	23'5'6	291.99	0.00	0	0
55.95	7.75E-02	22	234'	257.537	0.03	0.00669575	0.669575
56.075		C49	22'45'	291.99	0.00	0	0
56.2		38	345	257.537	0.00	0	0
56.213	3.21E-02	C47,75	22'44' 244'6	291.99	0.01	0.002447382	0.244738
56.365	7.14E-02	C48	22'45	291.99	0.02	0.005441098	0.54411
56.629		65	2356	291.99	0.00	0	0
56.871		C62	2346	291.99	0.00	0	0
57.124		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.563	2.98E-01	C44	22'35'	291.99	0.10	0.022691708	2.269171
57.887	5.62E-02	C37,42	344' 22'34'	291.99	0.02	0.004279755	0.427976
58.096	5.19E-03	72	23'55'	291.99	0.00	0.000395082	0.039508
58.557		68	23'45'	291.99	0.00	0	0
58.675	1.14E-01	C41,64,71	22'34 234'6 23'4'	291.99	0.04	0.008714776	0.871478
59.267		C103	22'45'6	326.44	0.00	0	0
59.46		57	233'5	291.99	0.00	0	0
59.547	5.78E-02	40	22'33'	291.99	0.02	0.004403042	0.440304

59.893	1.11E-02	C67,100	23'45 22'44'6	309.22	0.00	0.000796366	0.079637
60.29	9.52E-01	C63	234'5	291.99	0.33	0.07250313	7.250313
60.734		C74,94	244'5 22'35	326.44	0.00	0	0
60.882	5.56654	61	2345	291.99	1.91	0.424048525	42.40485
61.097	5.51E-01	C70	23'4'5	291.99	0.19	0.042008345	4.200835
61.312		98	22'346	326.44	0.00	0	0
61.452	1.76E-01	C102	22'456'	326.44	0.05	0.011988146	1.198815
61.477		66	23'44'	291.99	0.00	0	0
61.604	3.46E-01	93, 95	22'356 22'35'6	326.44	0.11	0.02357434	2.357434
62.178	1.17E-01	91	22'34'6	326.44	0.04	0.008002227	0.800223
62.303		C55	233'4	291.99	0.00	0	0
63.072	3.82E-01	C56,C60	233'4' 2344'	291.99	0.13	0.029138053	2.913805
63.583	3.80E-01	101	22'455'	326.44	0.12	0.025912048	2.591205
63.6		C90	22'34'5	326.44	0.00	0	0
63.826	3.86E-03	113	233'5'6	326.44	0.00	0.000262776	0.026278
64.058	0	99	22'44' 5	326.44	0.00	0	0
64.158		79	33'45'	291.99	0.00	0	0
64.915	1.22E-03	112	233'56	326.44	0.00	8.31798E-05	0.008318
65.148	1.15E-02	C78,83	33'45 22'33'5	326.44	0.00	0.000782752	0.078275
65.61	2.66E-02	C97	22'3'45	326.44	0.01	0.001813809	0.181381
65.759	2.44E-03	86	22'345	326.44	0.00	0.000166572	0.016657
65.952		C81,87	344'5 22'345'	326.44	0.00	0	0
66.074	1.36E-02	115	2344'6	326.44	0.00	0.000923322	0.092332
66.14		145	22'3466'	360.88	0.00	0	0
66.19		120	23'455'	326.44	0.00	0	0
66.459	2.97E-02	85	22'344'	326.44	0.01	0.002021428	0.202143
66.8		C136	22'33'66'	360.88	0.00	0	0
66.975	6.74E-02	C110,77	233'4'6 33'44'	309.22	0.02	0.004849009	0.484901
68.057	1.93E-02	C151	22'355'6	326.44	0.01	0.001311952	0.131195
68.45	7.60E-03	C124,135,1	2'3455' 22'33'56'	360.88	0.00	0.000468294	0.046829
68.703		C107,108	233'4'5 233'45'	326.44	0.00	0	0
68.759	6.77E-01	139, 149	22'344'6 22'34'	360.88	0.19	0.041730128	4.173013
69.193	2.61E-02	106	233'45	326.44	0.01	0.001776101	0.17761
69.855		133	22'33'55'	360.88	0.00	0	0
70.1		C134	22'33'56	360.88	0.00	0	0
70.242	1.61E-02	C114,131	2344'5 22'33'46	360.88	0.00	0.000992465	0.099247
70.507	4.47E-04	C165	233'55'6	360.88	0.00	2.7548E-05	0.002755
70.645	8.80E-03	C146	22'34'55'	360.88	0.00	0.000542103	0.05421
70.725		C161	233'45'6	360.88	0.00	0	0
70.881		184	22'344'66'	395.33	0.00	0	0
71.152	1.84E-02	153	22'44'55'	360.88	0.01	0.001134604	0.11346
71.35		168	23'44'5'6	360.88	0.00	0	0
71.375		127	33'455'	326.44	0.00	0	0
71.633	1.44E-02	132, 105	22'33'46'	360.88	0.00	0.000888805	0.088881
71.845		179	22'33'566'	395.33	0.00	0	0
72.647	7.64E-03	141	22'3455'	360.88	0.00	0.000470785	0.047078
73.207	3.57E-03	137, 176, 1	22'344'5 22'33'	360.88	0.00	0.000219933	0.021993
73.525		164	233'4'5'6	360.88	0.00	0	0
73.664	6.70E-03	163, 138	233'4'56 22344	360.88	0.00	0.000412897	0.04129
73.789		C158	233'44'6	360.88	0.00	0	0
73.881		160	234'456	360.88	0.00	0	0
74.188		186	22'34566'	395.33	0.00	0	0

74.427		C126,129,1	33'44'5 22'33'45	360.88	0.00	0	0
74.695		C175	22'33'45'6	395.33	0.00	0	0
75.05		159	233'455'	360.88	0.00	0	0
75.127		186, 182	22'34566' 22'344	395.33	0.00	0	0
75.55		C183	22'344'5'6	395.33	0.00	0	0
76.082		C167	23'44'55'	360.88	0.00	0	0
76.136		C128	22'33'44'	360.88	0.00	0	0
76.53		185	22'3455'6	395.33	0.00	0	0
77.088		C174	22'33'456'	395.33	0.00	0	0
77.222		181	22'344'56	395.33	0.00	0	0
77.661		C177	22'33'4'56	395.33	0.00	0	0
77.913		202, 171	22'33'55'66' 22'	429.78	0.00	0	0
78.09			233'44'5	360.88	0.00	0	0
78.481		C173	22'33'456	395.33	0.00	0	0
78.857		C197	22'33'44'66'	395.33	0.00	0	0
78.92		C192	233'455'6	395.33	0.00	0	0
79.472		C180	22'344'55'	395.33	0.00	0	0
79.755		C193	233'4'55'6	395.33	0.00	0	0
80.013		C191	233'44'5'6	395.33	0.00	0	0
80.646		C199	22'33'4566'	429.78	0.00	0	0
81.105		169	33'44'55'	360.88	0.00	0	0
82.003		C170,190	22'33'44'5 233'44'5	395.33	0.00	0	0
82.434		198	22'33'455'6	429.78	0.00	0	0
82.761		201	22'33'45'66'	429.78	0.00	0	0
82.969		C196,203	22'344'55'6 22'3:	429.78	0.00	0	0
85.437		C195,208	22'33'455'66'	464.23	0.00	0	0
86.285		C207	22'33'44'566'	464.23	0.00	0	0
87.325		C194	22'33'44'55'	429.78	0.00	0	0
87.737		C205	233'44'55'6'	429.78	0.00	0	0
90.39		C206	22'33'44'55'6	464.23	0.00	0	0
92.672		C209	22'33'44'55'66'	498.68	0.00	0	0
Total	12.87733				**4.50**	**1.00**	**100.00**

D135B

RetTime	Amount	Name	Configuration	lolecular weigl	Rel mol pre:	Mol%	In %
[min]	[ug/L]				(mass%/mo	rel mol pres/total mol pres	
33.323		C1	2	188.631	0.00	0	0
37.054	3.49E-01	3	4	188.631	0.18	0.007230053	0.723005
39.116	3.95609	C4,10	22' 26	223.084	1.77	0.069329376	6.932938
41.643	4.94E-01	C7,9	24 25	223.084	0.22	0.008656196	0.86562
42.938	4.42E-01	C6	23'	223.084	0.20	0.007743842	0.774384
43.432	1.43741	C5,8	23 24'	223.084	0.64	0.02519021	2.519021
44.852	8.21E-02	C14	35	223.084	0.04	0.001438016	0.143802
45.661		C19	22'6	257.537	0.00	0	0
47.072	1.66881	C11	33'	223.084	0.75	0.029245431	2.924543
47.317		12, 13	34, 34'	223.084	0.00	0	0
47.785	1.11E-01	C18	22'5	257.537	0.04	0.001689247	0.168925
48.126	16.74007	C15,17	44' 22'4	240.31	6.97	0.272335386	27.23354
48.807		C24,27	236 23'6	257.537	0.00	0	0
49.657	7.54E-02	C16,32	22'3 24'6	257.537	0.03	0.001144399	0.11444
50.522	9.90E-01	23	235	257.537	0.38	0.015031245	1.503124
50.888	1.06E-02	29	245	257.537	0.00	0.000160621	0.016062
51.097	1.50E-01	C54	22'66'	291.99	0.05	0.002002742	0.200274
51.329	3.77E-02	C26	23'5	257.537	0.01	0.000572635	0.057264
51.568	8.42E-02	C25	23'4	257.537	0.03	0.001278244	0.127824
52.106	1.35E-01	C31	24'5	257.537	0.05	0.002047426	0.204743
52.185		50	22'46	291.99	0.00	0	0
52.252	2.14E-01	28	244'	257.537	0.08	0.003241795	0.32418
53.174	4.47E-02	C21,33	234 2'34	257.537	0.02	0.00067829	0.067829
53.365	1.81E-02	53	22'56'	291.99	0.01	0.000241675	0.024167
53.808	1.03E-02	C51	22'46'	291.99	0.00	0.000137407	0.013741
53.977	4.75E-02		33'5	257.537	0.02	0.000720857	0.072086
54.531	3.67E-02		22'36	291.99	0.01	0.000490928	0.049093
54.988			34'5	257.537	0.00	0	0
55.341	1.34E-01	C46	22'36'	291.99	0.05	0.001799496	0.17995
55.534	9.78E-02	52,	22'55'	291.99	0.03	0.001309997	0.131
55.607		C73	23'5'6	291.99	0.00	0	0
55.946	9.35E-02	22	234'	257.537	0.04	0.001419461	0.141946
56.075		C49	22'45'	291.99	0.00	0	0
56.207	8.98E-02	38	345	257.537	0.03	0.001363762	0.136376
56.225		C47,75	22'44' 244'6	291.99	0.00	0	0
56.364	8.97E-02	C48	22'45	291.99	0.03	0.001200538	0.120054
56.54	3.97E-03	65	2356	291.99	0.00	5.31661E-05	0.005317
56.916	1.57E-02	C62	2346	291.99	0.01	0.000210327	0.021033
57.124		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.555	4.16E-01	C44	22'35'	291.99	0.14	0.00556478	0.556478
57.877	6.72E-02	C37,42	344' 22'34'	291.99	0.02	0.000899729	0.089973
58.152	7.79E-03	72	23'55'	291.99	0.00	0.000104287	0.010429
58.557		68	23'45'	291.99	0.00	0	0
58.682	1.62E-01	C41,64,71	22'34 234'6 23'4'	291.99	0.06	0.002171432	0.217143
59.21	7.93E-02	C103	22'45'6	326.44	0.02	0.00094974	0.094974
59.46		57	233'5	291.99	0.00	0	0
59.546	2.21E-01	40	22'33'	291.99	0.08	0.002953597	0.29536

59.834		C67,100	23'45 22'44'6	309.22	0.00	0	0
60.275	23.91018	C63	234'5	291.99	8.19	0.32013593	32.01359
60.734		C74,94	244'5 22'35	326.44	0.00	0	0
60.874	11.62673	61	2345	291.99	3.98	0.155671518	15.56715
61.091	7.63E-01	C70	23'4'5	291.99	0.26	0.010217602	1.02176
61.312		98	22'346	326.44	0.00	0	0
61.443	1.68E-01	C102	22'456'	326.44	0.05	0.002016336	0.201634
61.477		66	23'44'	291.99	0.00	0	0
61.603	3.54E-01	93, 95	22'356 22'35'6	326.44	0.11	0.004244589	0.424459
62.173	1.24E-01	91	22'34'6	326.44	0.04	0.001487758	0.148776
62.303		C55	233'4	291.99	0.00	0	0
63.063	3.58E-01	C56,C60	233'4' 2344'	291.99	0.12	0.004794933	0.479493
63.58	4.02E-01	101	22'455'	326.44	0.12	0.004811155	0.481116
63.6		C90	22'34'5	326.44	0.00	0	0
63.818	1.36E-02	113	233'5'6	326.44	0.00	0.000163278	0.016328
64.053	1.52E-02	99	22'44' 5	326.44	0.00	0.000182445	0.018245
64.158		79	33'45'	291.99	0.00	0	0
64.892	1.42E-02	112	233'56	326.44	0.00	0.000169841	0.016984
65.143	4.91E-02	C78,83	33'45 22'33'5	326.44	0.02	0.000588194	0.058819
65.607	1.40E-01	C97	22'3'45	326.44	0.04	0.001681843	0.168184
65.753	1.05E-02	86	22'345	326.44	0.00	0.000125972	0.012597
65.952		C81,87	344'5 22'345'	326.44	0.00	0	0
66.07	7.21E-02	115	2344'6	326.44	0.02	0.00086339	0.086339
66.14		145	22'3466'	360.88	0.00	0	0
66.19		120	23'455'	326.44	0.00	0	0
66.455	1.48E-01	85	22'344'	326.44	0.05	0.001770382	0.177038
66.8		C136	22'33'66'	360.88	0.00	0	0
66.968	3.68E-01	C110,77	233'4'6 33'44'	309.22	0.12	0.004650114	0.465011
68.056	1.42E-01	C151	22'355'6	326.44	0.04	0.001696226	0.169623
68.455	5.43E-02	C124,135,14	2'3455' 22'33'56'	360.88	0.02	0.000588309	0.058831
68.703		C107,108	233'4'5 233'45'	326.44	0.00	0	0
68.751	6.89E-01	139, 149	22'344'6 22'34'	360.88	0.19	0.007465819	0.746582
69.187	1.46E-01	106	233'45	326.44	0.04	0.001746598	0.17466
69.863	3.39E-03	133	22'33'55'	360.88	0.00	3.67747E-05	0.003677
70.114	1.57E-02	C134	22'33'56	360.88	0.00	0.000170607	0.017061
70.23	8.07E-02	C114,131	2344'5 22'33'46	360.88	0.02	0.000874376	0.087438
70.49	2.42E-03	C165	233'55'6	360.88	0.00	2.62026E-05	0.00262
70.642	5.22E-02	C146	22'34'55'	360.88	0.01	0.000565934	0.056593
70.725		C161	233'45'6	360.88	0.00	0	0
70.849	2.58E-03	184	22'344'66'	395.33	0.00	2.55306E-05	0.002553
71.148	9.87E-02	153	22'44'55'	360.88	0.03	0.001068837	0.106884
71.35		168	23'44'5'6	360.88	0.00	0	0
71.375		127	33'455'	326.44	0.00	0	0
71.621	7.14E-02	132, 105	22'33'46'	360.88	0.02	0.000773288	0.077329
71.845		179	22'33'566'	395.33	0.00	0	0
72.638	2.77E-02	141	22'3455'	360.88	0.01	0.000300082	0.030008
73.2	1.14E-02	137, 176, 13	22'344'5 22'33'	360.88	0.00	0.000123227	0.012323
73.462	2.64E-03	164	233'4'5'6	360.88	0.00	2.86185E-05	0.002862
73.66	3.03E-02	163, 138	233'4'56 22344	360.88	0.01	0.000327962	0.032796
73.831		C158	233'44'6	360.88	0.00	0	0
73.877		160	234'456	360.88	0.00	0	0
74.145		186	22'34566'	395.33	0.00	0	0

74.44		C126,129,17	33'44'5 22'33'45	360.88	0.00	0	0
74.779		C175	22'33'45'6	395.33	0.00	0	0
75.05		159	233'455'	360.88	0.00	0	0
75.11		186, 182	22'34566' 22'344	395.33	0.00	0	0
75.561		C183	22'344'5'6	395.33	0.00	0	0
76.082		C167	23'44'55'	360.88	0.00	0	0
76.123		C128	22'33'44'	360.88	0.00	0	0
76.489		185	22'3455'6	395.33	0.00	0	0
77.175		C174	22'33'456'	395.33	0.00	0	0
77.214		181	22'344'56	395.33	0.00	0	0
77.667		C177	22'33'4'56	395.33	0.00	0	0
77.9		202, 171	22'33'55'66' 22'	429.78	0.00	0	0
78.076			233'44'5	360.88	0.00	0	0
78.518		C173	22'33'456	395.33	0.00	0	0
78.857		C197	22'33'44'66'	395.33	0.00	0	0
78.92		C192	233'455'6	395.33	0.00	0	0
79.458		C180	22'344'55'	395.33	0.00	0	0
79.76		C193	233'4'55'6	395.33	0.00	0	0
80.01		C191	233'44'5'6	395.33	0.00	0	0
80.718		C199	22'33'4566'	429.78	0.00	0	0
81.115		169	33'44'55'	360.88	0.00	0	0
82.032		C170,190	22'33'44'5 233'44'5	395.33	0.00	0	0
82.468		198	22'33'455'6	429.78	0.00	0	0
82.739		201	22'33'45'66'	429.78	0.00	0	0
83.131		C196,203	22'344'55'6 22'33	429.78	0.00	0	0
85.702		C195,208	22'33'455'66'	464.23	0.00	0	0
86.319		C207	22'33'44'566'	464.23	0.00	0	0
87.195		C194	22'33'44'55'	429.78	0.00	0	0
87.736		C205	233'44'55'6'	429.78	0.00	0	0
90.35		C206	22'33'44'55'6	464.23	0.00	0	0
92.637		C209	22'33'44'55'66'	498.68	0.00	0	0
Total	68.5790334				**25.58**	**1.00**	**100.00**

D135C

RetTime [min]	Amount [ug/L]	Name	Configuration	Molecular	Rel mol pres (mass%/mo	Mol% rel mol pres/total mol pres	In %
33.323		C1	2	188.631	0.00	0	0
37.037		3	4	188.631	0.00	0	0
39.318		C4,10	22' 26	223.084	0.00	0	0
41.762		C7,9	24 25	223.084	0.00	0	0
42.881		C6	23'	223.084	0.00	0	0
43.542	1.05E-01	C5,8	23 24'	223.084	0.05	0.005277298	0.52773
44.806		C14	35	223.084	0.00	0	0
45.661		C19	22'6	257.537	0.00	0	0
47.075	1.72248	C11	33'	223.084	0.77	0.086563573	8.656357
47.317		12, 13	34, 34'	223.084	0.00	0	0
47.786	3.64E-02	C18	22'5	257.537	0.01	0.001586715	0.158672
48.147	6.59E-02	C15,17	44' 22'4	240.31	0.03	0.003073894	0.307389
48.807		C24,27	236 23'6	257.537	0.00	0	0
49.669	3.50E-02	C16,32	22'3 24'6	257.537	0.01	0.001522571	0.152257
50.514	3.17324	23	235	257.537	1.23	0.138137851	13.81379
50.87		29	245	257.537	0.00	0	0
51.082		C54	22'66'	291.99	0.00	0	0
51.327	1.61E-02	C26	23'5	257.537	0.01	0.000699909	0.069991
51.575		C25	23'4	257.537	0.00	0	0
52.114	5.93E-02	C31	24'5	257.537	0.02	0.002582604	0.25826
52.185		50	22'46	291.99	0.00	0	0
52.257	1.10E-01	28	244'	257.537	0.04	0.004805075	0.480507
53.248	1.18E-02	C21,33	234 2'34	257.537	0.00	0.000515851	0.051585
53.363	1.27E-02	53	22'56'	291.99	0.00	0.000487505	0.048751
53.812		C51	22'46'	291.99	0.00	0	0
53.998	3.57E-02		33'5	257.537	0.01	0.001554758	0.155476
54.543	4.18E-02		22'36	291.99	0.01	0.001605589	0.160559
54.997	1.07E-02		34'5	257.537	0.00	0.000466577	0.046658
55.326	2.69E-02	C46	22'36'	291.99	0.01	0.001032596	0.10326
55.533	8.16E-02	52,	22'55'	291.99	0.03	0.003131384	0.313138
55.607		C73	23'5'6	291.99	0.00	0	0
55.944	7.79E-02	22	234'	257.537	0.03	0.003389772	0.338977
56.075		C49	22'45'	291.99	0.00	0	0
56.205	6.92E-02	38	345	257.537	0.03	0.003010468	0.301047
56.225		C47,75	22'44' 244'6	291.99	0.00	0	0
56.364	8.00E-02	C48	22'45	291.99	0.03	0.003071022	0.307102
56.629		65	2356	291.99	0.00	0	0
56.871		C62	2346	291.99	0.00	0	0
57.124		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.559	3.58E-01	C44	22'35'	291.99	0.12	0.013742936	1.374294
57.88	6.18E-02	C37,42	344' 22'34'	291.99	0.02	0.002374549	0.237455
58.131		72	23'55'	291.99	0.00	0	0
58.557		68	23'45'	291.99	0.00	0	0
58.679	1.36E-01	C41,64,71	22'34 234'6 23'4'	291.99	0.05	0.005224911	0.522491
59.294	1.20E-02	C103	22'45'6	326.44	0.00	0.000413075	0.041307
59.46		57	233'5	291.99	0.00	0	0
59.546	7.99E-02	40	22'33'	291.99	0.03	0.003069037	0.306904
59.879	1.41E-02	C67,100	23'45 22'44'6	309.22	0.00	0.000511177	0.051118

60.287	2.45E-01	C63		234'5	291.99	0.08	0.009416712	0.941671
60.734		C74,94		244'5 22'35	326.44	0.00	0	0
60.875	13.24797		61	2345	291.99	4.54	0.508663722	50.86637
61.09	9.83E-01	C70		23'4'5	291.99	0.34	0.037737727	3.773773
61.312			98	22'346	326.44	0.00	0	0
61.443	2.06E-01	C102		22'456'	326.44	0.06	0.007071179	0.707118
61.477			66	23'44'	291.99	0.00	0	0
61.602	4.18E-01	93, 95		22'356 22'35'6	326.44	0.13	0.014341723	1.434172
62.172	1.48E-01		91	22'34'6	326.44	0.05	0.005082409	0.508241
62.303		C55		233'4	291.99	0.00	0	0
63.065	5.00E-01	C56,C60		233'4' 2344'	291.99	0.17	0.019184015	1.918401
63.578	5.22E-01		101	22'455'	326.44	0.16	0.017917786	1.791779
63.6		C90		22'34'5	326.44	0.00	0	0
63.813	2.46E-02		113	233'5'6	326.44	0.01	0.00084328	0.084328
64.053	1.02E-01		99	22'44' 5	326.44	0.03	0.00349199	0.349199
64.158			79	33'45'	291.99	0.00	0	0
64.906	1.63E-02		112	233'56	326.44	0.00	0.000558496	0.05585
65.137	7.04E-02	C78,83		33'45 22'33'5	326.44	0.02	0.002418113	0.241811
65.605	1.79E-01	C97		22'3'45	326.44	0.05	0.006156299	0.61563
65.749	1.53E-02		86	22'345	326.44	0.00	0.000525694	0.052569
65.952		C81,87		344'5 22'345'	326.44	0.00	0	0
66.068	9.58E-02		115	2344'6	326.44	0.03	0.003291077	0.329108
66.14			145	22'3466'	360.88	0.00	0	0
66.19			120	23'455'	326.44	0.00	0	0
66.453	2.50E-01		85	22'344'	326.44	0.08	0.008603042	0.860304
66.8		C136		22'33'66'	360.88	0.00	0	0
66.969	5.45E-01	C110,77		233'4'6 33'44'	309.22	0.18	0.019774141	1.977414
68.054	2.30E-01	C151		22'355'6	326.44	0.07	0.007888076	0.788808
68.444	8.32E-02	C124,135,144,		2'3455' 22'33'56'	360.88	0.02	0.002583947	0.258395
68.703		C107,108		233'4'5 233'45'	326.44	0.00	0	0
68.75	6.95E-01	139, 149		22'344'6 22'34'	360.88	0.19	0.021576611	2.157661
69.185	1.63E-01		106	233'45	326.44	0.05	0.005602783	0.560278
69.855			133	22'33'55'	360.88	0.00	0	0
70.1		C134		22'33'56	360.88	0.00	0	0
70.23	5.59E-02	C114,131		2344'5 22'33'46	360.88	0.02	0.00173684	0.173684
70.488	1.58E-03	C165		233'55'6	360.88	0.00	4.90335E-05	0.004903
70.642	3.30E-02	C146		22'34'55'	360.88	0.01	0.00102456	0.102456
70.725		C161		233'45'6	360.88	0.00	0	0
70.881			184	22'344'66'	395.33	0.00	0	0
71.148	8.57E-02		153	22'44'55'	360.88	0.02	0.002663656	0.266366
71.35			168	23'44'5'6	360.88	0.00	0	0
71.375			127	33'455'	326.44	0.00	0	0
71.623	7.42E-02	132, 105		22'33'46'	360.88	0.02	0.002304775	0.230477
71.845			179	22'33'566'	395.33	0.00	0	0
72.621	9.73E-03		141	22'3455'	360.88	0.00	0.000302234	0.030223
73.186	1.10E-02	137, 176, 130		22'344'5 22'33'	360.88	0.00	0.000343113	0.034311
73.525			164	233'4'5'6	360.88	0.00	0	0
73.659	3.21E-02	163, 138		233'4'56 22344	360.88	0.01	0.000996271	0.099627
73.831		C158		233'44'6	360.88	0.00	0	0
73.876			160	234'456	360.88	0.00	0	0
74.145			186	22'34566'	395.33	0.00	0	0
74.435		C126,129,178		33'44'5 22'33'45	360.88	0.00	0	0

74.732		C175		22'33'45'6	395.33	0.00	0		0	
75.05			159	233'455'	360.88	0.00	0		0	
75.104		186, 182		22'34566' 22'344	395.33	0.00	0		0	
75.553		C183		22'344'5'6	395.33	0.00	0		0	
76.082		C167		23'44'55'	360.88	0.00	0		0	
76.131		C128		22'33'44'	360.88	0.00	0		0	
76.409			185	22'3455'6	395.33	0.00	0		0	
77.175		C174		22'33'456'	395.33	0.00	0		0	
77.208			181	22'344'56	395.33	0.00	0		0	
77.665		C177		22'33'4'56	395.33	0.00	0		0	
77.9		202, 171		22'33'55'66' 22'	429.78	0.00	0		0	
78.074				233'44'5	360.88	0.00	0		0	
78.518		C173		22'33'456	395.33	0.00	0		0	
78.857		C197		22'33'44'66'	395.33	0.00	0		0	
78.92		C192		233'455'6	395.33	0.00	0		0	
79.442		C180		22'344'55'	395.33	0.00	0		0	
79.755		C193		233'4'55'6	395.33	0.00	0		0	
80.013		C191		233'44'5'6	395.33	0.00	0		0	
80.646		C199		22'33'4566'	429.78	0.00	0		0	
81.18			169	33'44'55'	360.88	0.00	0		0	
82.046		C170,190		22'33'44'5 233'44'5	395.33	0.00	0		0	
82.384			198	22'33'455'6	429.78	0.00	0		0	
82.743			201	22'33'45'66'	429.78	0.00	0		0	
83.147		C196,203		22'344'55'6 22'33	429.78	0.00	0		0	
85.732		C195,208		22'33'455'66'	464.23	0.00	0		0	
86.261		C207		22'33'44'566'	464.23	0.00	0		0	
87.27		C194		22'33'44'55'	429.78	0.00	0		0	
87.831		C205		233'44'55'6'	429.78	0.00	0		0	
90.359		C206		22'33'44'55'6	464.23	0.00	0		0	
92.629		C209		22'33'44'55'66'	498.68	0.00	0		0	
Total	25.4758859					**8.92**		**1.00**		**100.00**

D200A

RetTime [min]	Amount [ug/L]	Name		Configuration	Molecular	Rel mol pres (mass%/mo	Mol% rel mol pres/total mol pres	In %
33.076	7.91216	C1		2	188.631	4.19	0.301760862	30.17609
37.037			3	4	188.631	0.00	0	0
39.318		C4,10		22' 26	223.084	0.00	0	0
41.762		C7,9		24 25	223.084	0.00	0	0
42.93	4.72E-01	C6		23'	223.084	0.21	0.015235392	1.523539
43.506	3.06577	C5,8		23 24'	223.084	1.37	0.098867161	9.886716
44.768	0.0799519	C14		35	223.084	0.04	0.002578347	0.257835
45.661		C19		22'6	257.537	0.00	0	0
47.04	7.24285	C11		33'	223.084	3.25	0.233572649	23.35726
47.317		12, 13		34, 34'	223.084	0.00	0	0
47.775		C18		22'5	257.537	0.00	0	0
48.101	9.01E-01	C15,17		44' 22'4	240.31	0.37	0.026963531	2.696353
48.951	2.78E-02	C24,27		236 23'6	257.537	0.01	0.000776546	0.077655
49.645	5.24E-02	C16,32		22'3 24'6	257.537	0.02	0.001464205	0.14642
50.491	0.872924		23	235	257.537	0.34	0.024384717	2.438472
50.87			29	245	257.537	0.00	0	0
51.082		C54		22'66'	291.99	0.00	0	0
51.28	4.80E-02	C26		23'5	257.537	0.02	0.001340276	0.134028
51.57	3.78E-02	C25		23'4	257.537	0.01	0.001055062	0.105506
52.091	0.0477981	C31		24'5	257.537	0.02	0.001335217	0.133522
52.185			50	22'46	291.99	0.00	0	0
52.231	0.0944388		28	244'	257.537	0.04	0.002638103	0.26381
53.211		C21,33		234 2'34	257.537	0.00	0	0
53.327	4.84E-02		53	22'56'	291.99	0.02	0.001191359	0.119136
53.812		C51		22'46'	291.99	0.00	0	0
53.983	7.19E-02			33'5	257.537	0.03	0.002009012	0.200901
54.517	4.55E-02			22'36	291.99	0.02	0.001120218	0.112022
54.988				34'5	257.537	0.00	0	0
55.325		C46		22'36'	291.99	0.00	0	0
55.502	7.32E-02	52,		22'55'	291.99	0.03	0.001804398	0.18044
55.607		C73		23'5'6	291.99	0.00	0	0
55.915	1.05E-01		22	234'	257.537	0.04	0.002924605	0.292461
56.075		C49		22'45'	291.99	0.00	0	0
56.177	0.098361		38	345	257.537	0.04	0.002747668	0.274767
56.225		C47,75		22'44' 244'6	291.99	0.00	0	0
56.327	9.07E-02	C48		22'45	291.99	0.03	0.002233688	0.223369
56.629			65	2356	291.99	0.00	0	0
56.853	0.00786831	C62		2346	291.99	0.00	0.000193863	0.019386
57.004		C35		33'4	257.537	0.00	0	0
57.19			104	22'466'	326.44	0.00	0	0
57.526	0.293142	C44		22'35'	291.99	0.10	0.007222557	0.722256
57.847	7.06E-02	C37,42		344' 22'34'	291.99	0.02	0.00173997	0.173997
58.131			72	23'55'	291.99	0.00	0	0
58.557			68	23'45'	291.99	0.00	0	0
58.681	0.280058	C41,64,71		22'34 234'6 23'4'	291.99	0.10	0.006900188	0.690019
59.267		C103		22'45'6	326.44	0.00	0	0
59.46			57	233'5	291.99	0.00	0	0
59.511	6.68E-02		40	22'33'	291.99	0.02	0.001646322	0.164632
59.842	1.89E-02	C67,100		23'45 22'44'6	309.22	0.01	0.000438584	0.043858

60.248	1.52E+00	C63	234'5	291.99	0.52	0.037564974	3.756497
60.734		C74,94	244'5 22'35	326.44	0.00	0	0
60.839		61	2345	291.99	0.00	0	0
61.053	2.19095	C70	23'4'5	291.99	0.75	0.053981557	5.398156
61.283		98	22'346	326.44	0.00	0	0
61.411	0.31231	C102	22'456'	326.44	0.10	0.006882773	0.688277
61.477		66	23'44'	291.99	0.00	0	0
61.566	0.502334	93, 95	22'356 22'35'6	326.44	0.15	0.011070574	1.107057
62.137	0.26108	91	22'34'6	326.44	0.08	0.005753752	0.575375
62.303		C55	233'4	291.99	0.00	0	0
63.032	0.741898	C56,C60	233'4' 2344'	291.99	0.25	0.018279198	1.82792
63.542	0.736537	101	22'455'	326.44	0.23	0.016232003	1.6232
63.6		C90	22'34'5	326.44	0.00	0	0
63.769	1.30E-02	113	233'5'6	326.44	0.00	0.000287461	0.028746
64.018	0.261753	99	22'44' 5	326.44	0.08	0.005768584	0.576858
64.198	2.35E-02	79	33'45'	291.99	0.01	0.000578003	0.0578
64.9		112	233'56	326.44	0.00	0	0
65.101	9.90E-02	C78,83	33'45 22'33'5	326.44	0.03	0.002182205	0.218221
65.573	2.95E-01	C97	22'3'45	326.44	0.09	0.006503582	0.650358
65.727	1.61E-02	86	22'345	326.44	0.00	0.000355186	0.035519
65.952		C81,87	344'5 22'345'	326.44	0.00	0	0
66.039	0.171035	115	2344'6	326.44	0.05	0.003769316	0.376932
66.14		145	22'3466'	360.88	0.00	0	0
66.19		120	23'455'	326.44	0.00	0	0
66.42	3.96E-01	85	22'344'	326.44	0.12	0.008724379	0.872438
66.8		C136	22'33'66'	360.88	0.00	0	0
66.933	0.91033	C110,77	233'4'6 33'44'	309.22	0.29	0.021179671	2.117967
68.019	3.62E-01	C151	22'355'6	326.44	0.11	0.007987111	0.798711
68.414	1.58E-01	C124,135,144,	2'3455' 22'33'56'	360.88	0.04	0.003149086	0.314909
68.713	0.0661947	C107,108	233'4'5 233'45'	326.44	0.02	0.001458817	0.145882
68.965	6.89E-01	139, 149	22'344'6 22'34'	360.88	0.19	0.013732503	1.37325
69.148	3.55E-01	106	233'45	326.44	0.11	0.007822793	0.782279
69.855		133	22'33'55'	360.88	0.00	0	0
70.1		C134	22'33'56	360.88	0.00	0	0
70.193	2.74E-01	C114,131	2344'5 22'33'46	360.88	0.08	0.005466719	0.546672
70.455	0.00487321	C165	233'55'6	360.88	0.00	9.71479E-05	0.009715
70.588	8.08E-02	C146	22'34'55'	360.88	0.02	0.001611612	0.161161
70.725		C161	233'45'6	360.88	0.00	0	0
70.811		184	22'344'66'	395.33	0.00	0	0
71.116	2.25E-01	153	22'44'55'	360.88	0.06	0.004492232	0.449223
71.35		168	23'44'5'6	360.88	0.00	0	0
71.375		127	33'455'	326.44	0.00	0	0
71.586	0.185429	132, 105	22'33'46'	360.88	0.05	0.003696543	0.369654
71.838	8.53E-02	179	22'33'566'	395.33	0.02	0.001552158	0.155216
72.621	0.141157	141	22'3455'	360.88	0.04	0.002813977	0.281398
73.157	3.36E-02	137, 176, 130	22'344'5 22'33'	360.88	0.01	0.00066898	0.066898
73.466	0.0132299	164	233'4'5'6	360.88	0.00	0.000263739	0.026374
73.619	9.68E-02	163, 138	233'4'56 22344	360.88	0.03	0.001928865	0.192887
73.831		C158	233'44'6	360.88	0.00	0	0
73.842		160	234'456	360.88	0.00	0	0
74.025		186	22'34566'	395.33	0.00	0	0
74.398		C126,129,178	33'44'5 22'33'45	360.88	0.00	0	0

	74.732		C175	22'33'45'6	395.33	0.00	0	0
	75.05		159	233'455'	360.88	0.00	0	0
	75.07		186, 182	22'34566' 22'344	395.33	0.00	0	0
	75.502		C183	22'344'5'6	395.33	0.00	0	0
	76.088		C167	23'44'55'	360.88	0.00	0	0
	76.106		C128	22'33'44'	360.88	0.00	0	0
	76.398		185	22'3455'6	395.33	0.00	0	0
	77.186		C174	22'33'456'	395.33	0.00	0	0
	77.324		181	22'344'56	395.33	0.00	0	0
	77.629		C177	22'33'4'56	395.33	0.00	0	0
	77.9		202, 171	22'33'55'66' 22'	429.78	0.00	0	0
	78.04			233'44'5	360.88	0.00	0	0
	78.518		C173	22'33'456	395.33	0.00	0	0
	78.857		C197	22'33'44'66'	395.33	0.00	0	0
	78.994		C192	233'455'6	395.33	0.00	0	0
	79.41		C180	22'344'55'	395.33	0.00	0	0
	79.858		C193	233'4'55'6	395.33	0.00	0	0
	79.945		C191	233'44'5'6	395.33	0.00	0	0
	80.597		C199	22'33'4566'	429.78	0.00	0	0
	80.991		169	33'44'55'	360.88	0.00	0	0
	81.997		C170,190	22'33'44'5 233'44'5	395.33	0.00	0	0
	82.441		198	22'33'455'6	429.78	0.00	0	0
	82.726		201	22'33'45'66'	429.78	0.00	0	0
	83.169		C196,203	22'344'55'6 22'33	429.78	0.00	0	0
	85.743		C195,208	22'33'455'66'	464.23	0.00	0	0
	86.172		C207	22'33'44'566'	464.23	0.00	0	0
	87.273		C194	22'33'44'55'	429.78	0.00	0	0
	87.82		C205	233'44'55'6'	429.78	0.00	0	0
	90.393		C206	22'33'44'55'6	464.23	0.00	0	0
	92.617		C209	22'33'44'55'66'	498.68	0.00	0	0
Total		33.3516227				**13.90**	**1.00**	**100.00**

D200

RetTime [min]	Amount [ug/L]	Name	Configuration	Molecular	Rel mol pres (mass%/mo	Mol% rel mol pres/total mol pres	In %
33.081	7.75433	C1	2	188.631	4.11	0.432818719	43.28187
37.037		3	4	188.631	0.00	0	0
39.318		C4,10	22' 26	223.084	0.00	0	0
41.803	9.35E-02	C7,9	24 25	223.084	0.04	0.004411998	0.4412
42.881		C6	23'	223.084	0.00	0	0
43.623	1.19487	C5,8	23 24'	223.084	0.54	0.056393241	5.639324
44.813	1.05E-01	C14	35	223.084	0.05	0.00495616	0.495616
45.661		C19	22'6	257.537	0.00	0	0
47.051	3.02115	C11	33'	223.084	1.35	0.142586592	14.25866
47.317		12, 13	34, 34'	223.084	0.00	0	0
47.775		C18	22'5	257.537	0.00	0	0
48.108	1.3382	C15,17	44' 22'4	240.31	0.56	0.058630433	5.863043
48.807		C24,27	236 23'6	257.537	0.00	0	0
49.701	4.92E-02	C16,32	22'3 24'6	257.537	0.02	0.002011306	0.201131
50.498	5.16E-01	23	235	257.537	0.20	0.02110311	2.110311
50.854	1.22E-02	29	245	257.537	0.00	0.000499541	0.049954
51.082		C54	22'66'	291.99	0.00	0	0
51.323		C26	23'5	257.537	0.00	0	0
51.575		C25	23'4	257.537	0.00	0	0
52.119		C31	24'5	257.537	0.00	0	0
52.185		50	22'46	291.99	0.00	0	0
52.24	5.93E-02	28	244'	257.537	0.02	0.002423948	0.242395
53.242	9.44E-03	C21,33	234 2'34	257.537	0.00	0.000385956	0.038596
53.338		53	22'56'	291.99	0.00	0	0
53.812		C51	22'46'	291.99	0.00	0	0
53.996	4.67E-02		33'5	257.537	0.02	0.001910229	0.191023
54.517	8.22E-02		22'36	291.99	0.03	0.002962341	0.296234
54.975	5.24E-02		34'5	257.537	0.02	0.002143777	0.214378
55.302	5.43E-02	C46	22'36'	291.99	0.02	0.001956844	0.195684
55.513	5.69E-02	52,	22'55'	291.99	0.02	0.002051627	0.205163
55.607		C73	23'5'6	291.99	0.00	0	0
55.921	6.35E-02	22	234'	257.537	0.02	0.002597387	0.259739
56.075		C49	22'45'	291.99	0.00	0	0
56.186	5.80E-02	38	345	257.537	0.02	0.002370756	0.237076
56.225		C47,75	22'44' 244'6	291.99	0.00	0	0
56.334	7.77E-02	C48	22'45	291.99	0.03	0.002800925	0.280093
56.629		65	2356	291.99	0.00	0	0
56.854	1.64E-02	C62	2346	291.99	0.01	0.000591607	0.059161
57.004		C35	33'4	257.537	0.00	0	0
57.19		104	22'466'	326.44	0.00	0	0
57.535	2.05E-01	C44	22'35'	291.99	0.07	0.007375248	0.737525
57.858	4.06E-02	C37,42	344' 22'34'	291.99	0.01	0.001465725	0.146572
58.091	9.25E-03	72	23'55'	291.99	0.00	0.000333716	0.033372
58.557		68	23'45'	291.99	0.00	0	0
58.693	2.26E-01	C41,64,71	22'34 234'6 23'4'	291.99	0.08	0.008148052	0.814805
59.267		C103	22'45'6	326.44	0.00	0	0
59.46		57	233'5	291.99	0.00	0	0
59.518	1.25E-01	40	22'33'	291.99	0.04	0.004519636	0.451964

59.82	2.06E-02	C67,100	23'45 22'44'6	309.22	0.01	0.000701099	0.07011
60.256	1.88053	C63	234'5	291.99	0.64	0.067808964	6.780896
60.734		C74,94	244'5 22'35	326.44	0.00	0	0
60.848		61	2345	291.99	0.00	0	0
61.046	9.02E-01	C70	23'4'5	291.99	0.31	0.032509778	3.250978
61.283		98	22'346	326.44	0.00	0	0
61.421	1.15E-01	C102	22'456'	326.44	0.04	0.003703559	0.370356
61.477		66	23'44'	291.99	0.00	0	0
61.578	2.90E-01	93, 95	22'356 22'35'6	326.44	0.09	0.009339336	0.933934
62.148	6.94E-02	91	22'34'6	326.44	0.02	0.002238865	0.223887
62.303		C55	233'4	291.99	0.00	0	0
63.045	2.99E-01	C56,C60	233'4' 2344'	291.99	0.10	0.010775341	1.077534
63.552	3.52E-01	101	22'455'	326.44	0.11	0.011367637	1.136764
63.6		C90	22'34'5	326.44	0.00	0	0
63.765	1.48E-02	113	233'5'6	326.44	0.00	0.000476936	0.047694
64.025	3.30E-02	99	22'44' 5	326.44	0.01	0.001063401	0.10634
64.158		79	33'45'	291.99	0.00	0	0
64.9		112	233'56	326.44	0.00	0	0
65.117	6.57E-02	C78,83	33'45 22'33'5	326.44	0.02	0.002118706	0.211871
65.577	2.22E-01	C97	22'3'45	326.44	0.07	0.007171767	0.717177
65.759		86	22'345	326.44	0.00	0	0
65.926	3.93E-02	C81,87	344'5 22'345'	326.44	0.01	0.001267805	0.12678
66.047	7.53E-02	115	2344'6	326.44	0.02	0.00242932	0.242932
66.14		145	22'3466'	360.88	0.00	0	0
66.19		120	23'455'	326.44	0.00	0	0
66.435	2.49E-01	85	22'344'	326.44	0.08	0.008018024	0.801802
66.8		C136	22'33'66'	360.88	0.00	0	0
66.943	4.83E-01	C110,77	233'4'6 33'44'	309.22	0.16	0.016445225	1.644523
68.027	2.78E-01	C151	22'355'6	326.44	0.09	0.008980521	0.898052
68.428	1.83E-01	C124,135,1	2'3455' 22'33'56'	360.88	0.05	0.00533195	0.533195
68.703		C107,108	233'4'5 233'45'	326.44	0.00	0	0
68.732	7.13E-01	139, 149	22'344'6 22'34'	360.88	0.20	0.020790714	2.079071
69.167	2.07E-01	106	233'45	326.44	0.06	0.006679907	0.667991
69.855		133	22'33'55'	360.88	0.00	0	0
70.1		C134	22'33'56	360.88	0.00	0	0
70.206	1.34E-01	C114,131	2344'5 22'33'46	360.88	0.04	0.003897849	0.389785
70.478	3.77E-03	C165	233'55'6	360.88	0.00	0.00011009	0.011009
70.591	5.29E-02	C146	22'34'55'	360.88	0.01	0.00154375	0.154375
70.725		C161	233'45'6	360.88	0.00	0	0
70.811		184	22'344'66'	395.33	0.00	0	0
71.122	1.13E-01	153	22'44'55'	360.88	0.03	0.00329725	0.329725
71.35		168	23'44'5'6	360.88	0.00	0	0
71.375		127	33'455'	326.44	0.00	0	0
71.602	6.75E-02	132, 105	22'33'46'	360.88	0.02	0.00196928	0.196928
71.852	3.27E-02	179	22'33'566'	395.33	0.01	0.000870194	0.087019
72.593		141	22'3455'	360.88	0.00	0	0
73.153	1.42E-02	137, 176, 1	22'344'5 22'33'	360.88	0.00	0.000414893	0.041489
73.525		164	233'4'5'6	360.88	0.00	0	0
73.628	4.21E-02	163, 138	233'4'56 2234	360.88	0.01	0.001228965	0.122897
73.831		C158	233'44'6	360.88	0.00	0	0
73.847		160	234'456	360.88	0.00	0	0
74.025		186	22'34566'	395.33	0.00	0	0

74.385	C126,129,1	33'44'5 22'33'45	360.88	0.00	0		0
74.732	C175	22'33'45'6	395.33	0.00	0		0
75.05	159	233'455'	360.88	0.00	0		0
75.08	186, 182	22'34566' 22'344	395.33	0.00	0		0
75.522	C183	22'344'5'6	395.33	0.00	0		0
76.082	C167	23'44'55'	360.88	0.00	0		0
76.096	C128	22'33'44'	360.88	0.00	0		0
76.489	185	22'3455'6	395.33	0.00	0		0
77.18	C174	22'33'456'	395.33	0.00	0		0
77.226	181	22'344'56	395.33	0.00	0		0
77.658	C177	22'33'4'56	395.33	0.00	0		0
77.94	202, 171	22'33'55'66' 22'	429.78	0.00	0		0
78.041		233'44'5	360.88	0.00	0		0
78.518	C173	22'33'456	395.33	0.00	0		0
78.857	C197	22'33'44'66'	395.33	0.00	0		0
78.92	C192	233'455'6	395.33	0.00	0		0
79.438	C180	22'344'55'	395.33	0.00	0		0
79.755	C193	233'4'55'6	395.33	0.00	0		0
80.013	C191	233'44'5'6	395.33	0.00	0		0
80.646	C199	22'33'4566'	429.78	0.00	0		0
81.105	169	33'44'55'	360.88	0.00	0		0
82.024	C170,190	22'33'44'5 233'44'5	395.33	0.00	0		0
82.425	198	22'33'455'6	429.78	0.00	0		0
82.733	201	22'33'45'66'	429.78	0.00	0		0
83.241	C196,203	22'344'55'6 22'3:	429.78	0.00	0		0
85.782	C195,208	22'33'455'66'	464.23	0.00	0		0
86.272	C207	22'33'44'566'	464.23	0.00	0		0
87.268	C194	22'33'44'55'	429.78	0.00	0		0
87.737	C205	233'44'55'6'	429.78	0.00	0		0
90.414	C206	22'33'44'55'6	464.23	0.00	0		0
92.605	C209	22'33'44'55'66'	498.68	0.00	0		0
Total 22.21777				**9.50**		**1.00**	**100.00**

D200B

RetTime [min]	Amount [ug/L]	Name	Configuration	Molecular	Rel mol pres (mass%/mo	Mol% rel mol pres/total mol pres	In %
33.077		C1	2	188.631	0.00	0	0
37.343		3	4	188.631	0.00	0	0
39.318		C4,10	22' 26	223.084	0.00	0	0
41.762		C7,9	24 25	223.084	0.00	0	0
42.763	2.74E-01	C6	23'	223.084	0.12	0.013362272	1.336227
43.539		C5,8	23 24'	223.084	0.00	0	0
44.789	4.28E-02	C14	35	223.084	0.02	0.002087166	0.208717
45.661		C19	22'6	257.537	0.00	0	0
47.048	2.89283	C11	33'	223.084	1.30	0.141136115	14.11361
47.317		12, 13	34, 34'	223.084	0.00	0	0
47.765	3.15E-02	C18	22'5	257.537	0.01	0.001331399	0.13314
48.105	2.53822	C15,17	44' 22'4	240.31	1.06	0.114958261	11.49583
48.807		C24,27	236 23'6	257.537	0.00	0	0
49.636	1.00E-01	C16,32	22'3 24'6	257.537	0.04	0.004240806	0.424081
50.493	2.14458	23	235	257.537	0.83	0.090632986	9.063299
50.76	1.79E-02	29	245	257.537	0.01	0.000755507	0.075551
51.082		C54	22'66'	291.99	0.00	0	0
51.309	6.75E-02	C26	23'5	257.537	0.03	0.002852743	0.285274
51.559	5.23E-02	C25	23'4	257.537	0.02	0.002211844	0.221184
52.088	1.38E-01	C31	24'5	257.537	0.05	0.005816819	0.581682
52.185		50	22'46	291.99	0.00	0	0
52.227	3.66E-01	28	244'	257.537	0.14	0.015465861	1.546586
53.224	1.92E-02	C21,33	234 2'34	257.537	0.01	0.000813029	0.081303
53.33	2.32E-02	53	22'56'	291.99	0.01	0.00086557	0.086557
53.784	1.37E-02	C51	22'46'	291.99	0.00	0.00051166	0.051166
53.978	6.86E-02		33'5	257.537	0.03	0.002900283	0.290028
54.51	7.06E-02		22'36	291.99	0.02	0.002632062	0.263206
54.95	2.24E-02		34'5	257.537	0.01	0.00094685	0.094685
55.3	5.23E-02	C46	22'36'	291.99	0.02	0.001950924	0.195092
55.505	2.30E-01	52,	22'55'	291.99	0.08	0.008572317	0.857232
55.607		C73	23'5'6	291.99	0.00	0	0
55.916	2.26E-01	22	234'	257.537	0.09	0.009543388	0.954339
56.075		C49	22'45'	291.99	0.00	0	0
56.179	2.17E-01	38	345	257.537	0.08	0.009153484	0.915348
56.225		C47,75	22'44' 244'6	291.99	0.00	0	0
56.332	2.10E-01	C48	22'45	291.99	0.07	0.007837593	0.783759
56.629		65	2356	291.99	0.00	0	0
56.871		C62	2346	291.99	0.00	0	0
57.004		C35	33'4	257.537	0.00	0	0
57.185	3.07E-02	104	22'466'	326.44	0.01	0.001023629	0.102363
57.528	8.72E-01	C44	22'35'	291.99	0.30	0.032497428	3.249743
57.85	1.50E-01	C37,42	344' 22'34'	291.99	0.05	0.005587087	0.558709
58.131		72	23'55'	291.99	0.00	0	0
58.557		68	23'45'	291.99	0.00	0	0
58.682	4.73E-01	C41,64,71	22'34 234'6 23'4'	291.99	0.16	0.017630846	1.763085
59.267		C103	22'45'6	326.44	0.00	0	0
59.46		57	233'5	291.99	0.00	0	0
59.516	1.45E-01	40	22'33'	291.99	0.05	0.005413759	0.541376
59.852	2.64E-02	C67,100	23'45 22'44'6	309.22	0.01	0.000927549	0.092755

60.254	3.04709	C63	234'5	291.99	1.04	0.113579767	11.35798
60.734		C74,94	244'5 22'35	326.44	0.00	0	0
60.846		61	2345	291.99	0.00	0	0
61.063	1.91754	C70	23'4'5	291.99	0.66	0.071475981	7.147598
61.283		98	22'346	326.44	0.00	0	0
61.415	5.76E-01	C102	22'456'	326.44	0.18	0.019193288	1.919329
61.477		66	23'44'	291.99	0.00	0	0
61.573	9.69E-01	93, 95	22'356 22'35'6	326.44	0.30	0.032308221	3.230822
62.145	3.23E-01	91	22'34'6	326.44	0.10	0.010770418	1.077042
62.303		C55	233'4	291.99	0.00	0	0
63.036	1.16521	C56,C60	233'4' 2344'	291.99	0.40	0.043433007	4.343301
63.55	1.08471	101	22'455'	326.44	0.33	0.036165456	3.616546
63.6		C90	22'34'5	326.44	0.00	0	0
63.777	1.85E-02	113	233'5'6	326.44	0.01	0.000616024	0.061602
64.023	4.68E-01	99	22'44' 5	326.44	0.14	0.015611217	1.561122
64.158		79	33'45'	291.99	0.00	0	0
64.886	1.73E-02	112	233'56	326.44	0.01	0.000575851	0.057585
65.114	8.53E-02	C78,83	33'45 22'33'5	326.44	0.03	0.002844788	0.284479
65.577	3.46E-01	C97	22'3'45	326.44	0.11	0.011538164	1.153816
65.723	1.71E-02	86	22'345	326.44	0.01	0.00056913	0.056913
65.919	5.05E-02	C81,87	344'5 22'345'	326.44	0.02	0.001683694	0.168369
66.044	1.79E-01	115	2344'6	326.44	0.05	0.005978198	0.59782
66.14		145	22'3466'	360.88	0.00	0	0
66.19		120	23'455'	326.44	0.00	0	0
66.427	4.60E-01	85	22'344'	326.44	0.14	0.015334952	1.533495
66.8		C136	22'33'66'	360.88	0.00	0	0
66.937	1.23378	C110,77	233'4'6 33'44'	309.22	0.40	0.043427101	4.34271
68.025	3.79E-01	C151	22'355'6	326.44	0.12	0.01264409	1.264409
68.417	1.50E-01	C124,135,14	2'3455' 22'33'56'	360.88	0.04	0.004530073	0.453007
68.719	7.99E-02	C107,108	233'4'5 233'45'	326.44	0.02	0.002665213	0.266521
68.944		139, 149	22'344'6 22'34'	360.88	0.00	0	0
69.159	5.31E-01	106	233'45	326.44	0.16	0.017715876	1.771588
69.805	7.95E-03	133	22'33'55'	360.88	0.00	0.000239632	0.023963
70.1		C134	22'33'56	360.88	0.00	0	0
70.197	3.60E-01	C114,131	2344'5 22'33'46	360.88	0.10	0.010851155	1.085116
70.472	5.56E-03	C165	233'55'6	360.88	0.00	0.000167624	0.016762
70.59	7.30E-02	C146	22'34'55'	360.88	0.02	0.002202239	0.220224
70.725		C161	233'45'6	360.88	0.00	0	0
70.811		184	22'344'66'	395.33	0.00	0	0
71.119	2.71E-01	153	22'44'55'	360.88	0.08	0.008184865	0.818486
71.35		168	23'44'5'6	360.88	0.00	0	0
71.375		127	33'455'	326.44	0.00	0	0
71.592	2.04E-01	132, 105	22'33'46'	360.88	0.06	0.006143534	0.614353
71.851	2.00E-02	179	22'33'566'	395.33	0.01	0.000551925	0.055192
72.609	6.28E-02	141	22'3455'	360.88	0.02	0.001893743	0.189374
73.167	1.21E-02	137, 176, 13	22'344'5 22'33'	360.88	0.00	0.000366278	0.036628
73.439	1.02E-02	164	233'4'5'6	360.88	0.00	0.000307356	0.030736
73.626	9.19E-02	163, 138	233'4'56 2234	360.88	0.03	0.002771899	0.27719
73.831		C158	233'44'6	360.88	0.00	0	0
73.849		160	234'456	360.88	0.00	0	0
74.025		186	22'34566'	395.33	0.00	0	0
74.406		C126,129,17	33'44'5 22'33'45	360.88	0.00	0	0

74.732		C175	22'33'45'6	395.33	0.00	0	0
75.05		159	233'455'	360.88	0.00	0	0
75.074		186, 182	22'34566' 22'344	395.33	0.00	0	0
75.508		C183	22'344'5'6	395.33	0.00	0	0
76.082		C167	23'44'55'	360.88	0.00	0	0
76.099		C128	22'33'44'	360.88	0.00	0	0
76.489		185	22'3455'6	395.33	0.00	0	0
77.176		C174	22'33'456'	395.33	0.00	0	0
77.226		181	22'344'56	395.33	0.00	0	0
77.648		C177	22'33'4'56	395.33	0.00	0	0
77.9		202, 171	22'33'55'66' 22'	429.78	0.00	0	0
78.046			233'44'5	360.88	0.00	0	0
78.518		C173	22'33'456	395.33	0.00	0	0
78.857		C197	22'33'44'66'	395.33	0.00	0	0
78.92		C192	233'455'6	395.33	0.00	0	0
79.425		C180	22'344'55'	395.33	0.00	0	0
79.697		C193	233'4'55'6	395.33	0.00	0	0
80.013		C191	233'44'5'6	395.33	0.00	0	0
80.646		C199	22'33'4566'	429.78	0.00	0	0
81.105		169	33'44'55'	360.88	0.00	0	0
82.027		C170,190	22'33'44'5 233'44'5	395.33	0.00	0	0
82.434		198	22'33'455'6	429.78	0.00	0	0
82.733		201	22'33'45'66'	429.78	0.00	0	0
83.179		C196,203	22'344'55'6 22'3	429.78	0.00	0	0
85.632		C195,208	22'33'455'66'	464.23	0.00	0	0
86.272		C207	22'33'44'566'	464.23	0.00	0	0
87.299		C194	22'33'44'55'	429.78	0.00	0	0
87.8		C205	233'44'55'6'	429.78	0.00	0	0
90.494		C206	22'33'44'55'6	464.23	0.00	0	0
92.674		C209	22'33'44'55'66'	498.68	0.00	0	0
Total	25.73274				**9.19**	**1.00**	**100.00**