The impact of oxygen exposure on long-chain alkyl diols and the long chain diol index (LDI) – a long-term incubation study

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In recent years, long chain alkyl diols (LCDs) have been used increasingly to study and reconstruct past sea surface temperatures using the long chain diol index (LDI), which is based on changes in the distribution of 1,15-LCDs. However, the impact of diagenesis on LCDs and the LDI is still poorly constrained. Here, we studied the impact of oxygen exposure on LCDs and the LDI by aerobically incubating biomass of the LCD-producing alga Nannochloropsis oculata for 271 days. The concentrations of extractable free- and bound, residually ester-bound and residually-bound glycocidic ether- or amide-bound saturated fatty acids and LCDs were determined. A significant impact of oxygen exposure was observed for C14, C16 and C18 saturated fatty acids and the C20:5 polyunsaturated fatty acid, as their concentration decreased significantly over time, irrespective of their mode of binding. LCDs, in contrast, increased significantly in concentration over incubation time, e.g. up to a 30-fold increase in concentrations for residually ester-bound LCDs at day 125 compared to concentrations at the beginning of the experiment. This increase in concentration most likely represents a release of LCDs from the insoluble biopolymer algaenan due to the impact of oxygen exposure. Values of the LDI differed strongly depending on the mode of occurrence of LCDs in the biomass. However, despite the large changes in concentration of LCDs in response to oxygen exposure, the LDI remained relatively stable after prolonged degradation. This suggests that oxygen exposure may not have a substantial impact on the LDI of extractable LCDs used for its determination.

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1. Introduction

Long chain alkyl diols (LCDs) were first discovered in sediments of the Black Sea (de Leeuw et al., 1981) and since then have been studied in a large variety of marine and freshwater environments (e.g. Versteegh et al., 1997; Sinninghe Damsté et al., 2003; Rampen et al., 2007, 2008, 2014a, 2014b; Zhang et al., 2011; Romero-Viana et al., 2012; de Bar et al., 2016; Lattaud et al., 2017a). They are composed of long n-alkyl chains with chain lengths of mainly C28 to C32 and contain alcohol groups at C1 and at a mid-chain position, most notably between positions C12 to C15 (Versteegh et al., 1997). Saturated and unsaturated C28 and C30 1,14-LCDs have been shown to be produced by Proboscia diatoms (Sinninghe Damsté et al., 2003; Rampen et al., 2007) and C29-C32 1,14-LCDs have also been identified in the Dictyochophycea Apedinella radians (Rampen et al., 2011). C28 to C32 1,13- and 1,15-LCDs have been identified in cultures of freshwater and marine Eustigmatophyte algae, e.g. Nannochloropsis oculata and Nannochloropsis oceanica (Volkman et al., 1992, 1999; Gelin et al., 1997; Mejanelle et al., 2003; Shimokawara et al., 2010; Rampen et al., 2014a; Balzano et al., 2017; Zhang and Volkman, 2017), but the diol distributions in marine environments are dissimilar to those of cultured algae (Volkman et al., 1992; Versteegh et al., 1997; Rampen et al., 2012, 2014a), suggesting unknown marine sources. LCDs in algal cell material of Eustigmatophytes and in the sediment have been shown to occur in different forms, i.e. as free-extractable, extractable-bound (released by hydrolysis of the extract) and residually-bound lipids (released by hydrolysis of the residual biomass left after extraction) (Gelin et al., 1997; Ahmed et al., 2000; Grossi et al., 2001; Hoefs et al., 2002; Shimokawara et al., 2010). Additionally, they are also thought to be the building blocks of the insoluble biopolymer algaenan, which occurs in the

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Several indices based on LCD distributions have been proposed to reconstruct past conditions, such as upwelling, oxic degradation and riverine input, as well as sea surface temperatures (SST). The long chain diol index (LDI) is based on the fractional abundances of C_{28} 1,13- and C_{30} 1,15-LCDs and correlates strongly with SST (Rampen et al., 2012). Upwelling indices use the relative abundances of C_{28} 1,14- to C_{28}/C_{30} 1,13- or C_{30} 1,15-LCDs (Rampen et al., 2008; Willmott et al., 2010). The diol oxidation index (DOXI), calculated as the ratio of 1,15-keto-ols versus LCDs, is used to study oxic degradation in sediments, thereby assuming that keto-ols are oxidative products of LCDs (Ferreira et al., 2001). Finally, the %C_{32} 1,15 of all LCDs (de Bar et al., 2016; Lattaud et al., 2017a, 2017b) is used to study riverine inputs into the marine environment. These indices have since shown promise in paleo-environmental reconstruction studies and have been applied in different marine environments (e.g. Pancost et al., 2009; Willmott et al., 2010; Rampen et al., 2012, 2014b; Seki et al., 2012; Lopes dos Santos et al., 2013; Rodrigo-Gámiz et al., 2014, 2015, 2016; Nieto-Moreno et al., 2015; Planqu et al., 2015).

Some uncertainties in the application of LCD indices still remain to be addressed. Only a few studies have considered the impact of diagenesis on the abundance and distribution of LCDs (Ferreira et al., 2001; Versteegh et al., 2010; Bogus et al., 2012). As shown by various studies, biomarker distributions can be altered substantially by oxygen exposure (Sun and Wakeham, 1994; Hoefs et al., 1998; Prabh et al., 2003; Rontani et al., 2009, 2013), but only Sinninghe Damsté et al. (2002), Hoefs et al. (2002) and Rodrigo-Gámiz et al. (2016) analyzed the impact of oxic degradation on LCD distributions. Sinninghe Damsté et al. (2002) analyzed sediment cores from the northern Arabian Sea, studying the impact of oxygen exposure time on the preservation potential of various biomarkers and finding relatively high preservation efficiencies of LCDs. Hoefs et al. (2002) studied free and bound lipid biomarkers in oxidized and un-oxidized parts of Madeira Abyssal Plain turbidites, finding higher preservation of bound LCDs compared to free LCDs. Analysis of Arabian Sea surface sediments deposited under contrasting redox conditions by Rodrigo-Gámiz et al. (2016) showed a strong decrease in concentration of LCDs with increasing oxygen exposure, as well as a slightly higher degradation rate of 1,15-LCDs compared to 1,14- and 1,13-LCDs. This resulted in a drop of the LDI from 0.94 to 0.85 with increasing water depth. Additionally, the DOXI ratios showed no distinctive trends over water depth and only slight increases in value with increasing oxygen concentration were observed. The impact of anoxic degradation on LCDs has been constrained by a few studies, most notably by Sun and Wakeham (1994) and Grossi et al. (2001). Sun and Wakeham (1994) reported a high level of resistance to anoxic degradation during early diagenesis and an almost 100% burial efficiency of LCDs in Black Sea sediments. Grossi et al. (2001) incubated Nannochloropsis salina anaerobically over 442 days, revealing short-term increases (degradation days 50–100) in LCD concentrations, which were proposed to be caused by a release of LCDs from the algalan. Following this, concentrations of LCDs decreased over time, albeit to a lesser extent than other associated compounds, such as alkenes, phytol, sterols and fatty acids. Both studies thus show relatively low degradation rates for LCDs under anoxic conditions.

Here we present a laboratory study analyzing the impact of oxygen exposure on LCD concentrations and distributions in Nannochloropsis oculata biomass, which was aerobically incubated for 271 days. The concentrations of free and bound LCDs and major other lipids were monitored over degradation time. Furthermore, the impact of oxic degradation on the LDI was constrained.

### 2. Material and methods

#### 2.1. Set up of aerobic biomass incubations

A large batch of the LCD-producing alga Nannochloropsis oculata, strain CCMP525, was ordered from Reed Mariculture Inc and kept frozen at −20 °C until the set-up of incubation. Defrosted biomass (20g) was transferred to Erlenmeyer flasks each containing 10 g of sediment and 210 ml of sea water, both collected from the Dutch Wadden Sea in March 2015 and acclimated after collecting for 24 h at 25 °C. Forty two Erlenmeyer flasks were prepared and placed into a shaker table in the dark, at 25 °C and stirred at 100 rpm for 271 days under aerobic conditions. Sea water levels were monitored weekly and bi-distilled water was added when required to keep the water volume constant and to counteract evaporation effects.

#### 2.2. Lipid analysis

At predetermined sampling days, three Erlenmeyer flasks were randomly selected. For each flask, the water layer was separated from the particulate matter by centrifugation at 3000 rpm for 120 min. The particulate matter was subsequently freeze dried and processed using the analytical scheme outlined in Fig. 1, which is an approach modified from Gelin et al. (1997) and Goossens et al. (1986).

The particulate matter was extracted ultrasonically with methanol/dichloromethane (MeOH/DCM) (2:1, v/v, 4x). DCM phases were combined and dried using rotary evaporation and drying under N₂. The extract resulting from this work-up step was labelled Extract 1 (E1).

An aliquot of E1 was subsequently saponified by refluxing with 1 M KOH in MeOH for 1 h. The DCM phase was separated from the H₂O/MeOH phase and dried under N₂. This fraction was subsequently acid hydrolyzed in a last step by refluxing with 1.5 M HCl in MeOH for 3 h. After cooling, the mixture was neutralized using 2 N KOH in MeOH and extracted with DCM/H₂O (1:1, v/v, 3x). The DCM phases were separated from the H₂O/MeOH phase and combined to be dried under N₂. The base and acid hydrolyzed E1 extract is termed Extract 2 (E2).

The residue obtained after ultrasonic extraction of the particulate phase of the incubation experiment was dried under N₂ and saponified by refluxing with 1 M KOH in MeOH for 1 h. The mixture was centrifuged and the liquid phase was collected into a separatory funnel and acidified to a pH of 3 using 2 N HCl in MeOH.
The residue was subsequently extracted using H2O/MeOH (1:1, v/v), MeOH (2x) and DCM (3x) and all solvents were combined into the separatory funnel. The DCM layer was separated from the MeOH/H2O layer and collected, the MeOH/H2O layer was extracted with DCM (3x) and the combined DCM phases were dried under N2. This extract was subsequently acid hydrolyzed using the conditions described above and yielded Extract 3 (E3). In a last step, the saponified residues were acid hydrolyzed by refluxing with 1.5 M HCl in MeOH for 3 h. Solvents were collected into a separatory funnel after cooling and neutralized using 2 N KOH in MeOH. Residues were subsequently extracted using H2O/MeOH (1:1, v/v), MeOH (2x) and DCM (3x) and solvents were collected into the separatory funnel. The DCM layer was separated from the MeOH/H2O layer and collected, the MeOH/H2O layer was extracted with DCM (3x) and the combined DCM phases were dried under N2. The extracted residue after base hydrolysis was subsequently also acid hydrolyzed following the procedures described above yielding Extract 4 (E4).

E1–E4 were eluted with DCM over a small Pasteur pipette containing Na2SO4 to remove remaining salts. An internal standard C22/Δ3,6-diepoxide was added to aliquots of E1–E4 which were subsequently methylated with diazomethane in diethylether. Very polar components were removed by eluting the methylated E1–E4 with ethyl acetate over a small Pasteur pipette containing silica gel.

Prior to analysis by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), E1–E4 were silylated by adding BSTFA [N,O-bis(trimethyl-silyl)trifluoroacetamide] and pyridine and heated up to 60 °C for 20 min. Subsequently, samples were dissolved in ethyl acetate.

2.3. GC/MS analysis of LCDs

Samples were analyzed for LCDs on an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass spectrometer, equipped with a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm). Samples were injected at 70 °C oven temperature, increasing to 130 °C at 20 °C/min and then 320 °C at 4 °C/min which was held for 25 min with a helium flow of 2 ml/min. The mass spectrometer operated with an ionization energy of 70 eV. LCDs were quantified by selective ion monitoring (SIM) of m/z 187 (characteristic for the internal standard), m/z 313 (characteristic for the C28 1,13- and C30 1,15-LCD), m/z 339 (characteristic for the C32:1 1,15-LCD) and m/z 341 (characteristic for the C32 1,15-LCD) (Versteegh et al., 1997; Rodrigo-Gámiz et al., 2015). Additional compounds, e.g. fatty acids, polyunsaturated fatty acids, phytol and sterols, were identified on the same instrument in full scan mode (m/z 50–800) and analyzed on an Agilent 7890B gas chromatograph with helium as the carrier gas at a flow of 2 ml/min using the same temperature program as that for GC/MS. Fatty acids were identified by comparison of mass spectra with published mass spectra and quantified by integration of respective peak areas of fatty acids and the internal standard. The calculated concentrations given in this paper were normalized on dry weight of the biomass, in mg/g or μg/g.

The LD1 was calculated as follows (Rampen et al., 2012):

$$\text{Long chain Diol Index (LDI)} = \frac{F_{c_{28}1.15-\text{diol}} + F_{c_{30}1.13-\text{diol}} + F_{c_{32}1.13-\text{diol}} + F_{c_{32}1.15-\text{diol}}}{F_{c_{28}1.13-\text{diol}}}$$

3. Results

Biomass of the LCD-producing alga *Nannochloropsis oculata* was incubated in the presence of sediment and water from the North Sea under aerobic conditions, for a period of 271 days. Four extracts (E1–E4), each representing lipids occurring in the biomass in a different way, were obtained (Fig. 1) following the work-up procedures of e.g. Gelin et al. (1997) or Goossens et al. (1986). E1 contains freely occurring and –extractable lipids, while E2 contains free-extractable lipids from E1 as well as extractable lipids which are ester-bound or bound by glycosidic ether- or amide bonds. E3 represents residually ester-bound lipids while E4 contains residually-bound glycosidic ether- or amide-bound lipids (Goossens et al., 1986, 1989). LCDs were detected in all fractions. Both E1 and E2 contained saturated and polyunsaturated fatty acids, phytol and sterols (predominantly cholesterol and minor amounts of C29Δ5,24-sterols). E3 contained saturated and polyunsaturated fatty acids, α-hydroxy and β-hydroxy fatty acids with chain lengths of C24 to C26 and C17 to C18, respectively and to a lesser extent sterols, predominantly cholesterol. E4, was mostly comprised of α- and β-hydroxy fatty acids with chain lengths of C24 to C26 and C17 to C18, respectively and to a lesser extent, of saturated fatty acids. Long chain keto-ols were not detected and mid-chain hydroxy methyl alkanoates were only present in trace amounts. This lipid composition is in good agreement with previous studies of the lipids of *Nannochloropsis* species (Volkman et al., 1992, 1993; Olofsson et al., 2012; Mitra et al., 2015; Balzano et al., 2017; Zhang and Volkman, 2017). Analysis of the sediment and sea water from the Dutch Wadden Sea used, did not show the presence of LCDs. Furthermore, saturated fatty acids were present in low abundances (up to 9% of total fatty acids in the experiment) and the C20:5 PUFA was not detected; therefore both are ignored here. Additionally, the pure biomass of strain CCMP5525 was analyzed to confirm that the reported LCDs in this study were solely derived from this alga.

In this study, we mainly focus on LCDs, as well as the dominant saturated fatty acids and the C20:5 polyunsaturated fatty acid (PUFA), to contrast their different responses to oxygen exposure.

3.1. Fatty acids

C14, C16 and C18 saturated fatty acids were identified in E1–E4. The concentrations of summed saturated fatty acids (C14 + C16 + C18) were highest in E1 and E3 with 7.9 ± 1.4 and 10.1 ± 1.6 mg/g respectively (Fig. 2 A). The concentration of free-extractable saturated fatty acids (E1) decreased by 98% during the first 35 days of incubation and stayed low at 0.2 ± 0.1 mg/g throughout the remainder of the experiment. Free- and extractable-bound (E2) saturated fatty acids initially increased during the first 3 days of incubation up to 30.7 ± 8.3 mg/g and subsequently showed a continuous slow decrease until reaching a plateau of 6.1 ± 0.7 mg/g at 169 incubation days for the remainder of the experiment. The concentration of residually ester-bound (E3) saturated fatty acids decreased slowly, reaching concentrations of 2.9 ± 0.1 mg/g by the end of the experiment. Residually-bound glycosidic ether- or amide-bound (E4) fatty acids stayed low in concentration at 0.3 ± 0.1 mg/g throughout the experiment.

The C20:5 PUFA is also a prominent lipid, in addition to saturated fatty acids, in *N. oculata* biomass. In this incubation study this fatty acid occurred in the free-extractable (E1) and free- and extractable-bound (E2) extracts, but was not identified in E3 and E4 (Fig. 2 B). Its concentration in the free-extractable (E1) form decreased rapidly during the first 15 days of incubation, from initial values of 18.0 ± 1.7 mg/g to below detection limits. The concentration of free- and extractable-bound (E2) C20:5 PUFA decreased rapidly from 49.8 ± 2.0 mg/g to 12.9 ± 1.4 mg/g during the first 3 days and stayed approximately constant until 35 days. Subsequently, values slowly decreased until the end of incubation time to concentrations of 0.7 ± 0.1 mg/g. The residually ester-bound C20:5 PUFA was first detected at day 22 of incubation at
Fig. 2. Concentrations of lipids over time in an incubation experiment where biomass of the Eustigmatophyte alga *Nannochloropsis oculata* was exposed to oxygen in the presence of sediment and sea water: (A) concentration of the sum of saturated fatty acids, (B) concentration of C<sub>20:5</sub> PUFA, (C) concentration of the sum of LCDs. Concentrations are given over degradation time for free-extractable (E1), free- and extractable-bound (E2), residually ester-bound (E3) and residually-bound glycosidic ether- or amide-bound compounds (E4). Error bars represent standard deviations calculated from triplicate incubations.
3.2 ± 2.1 mg/g, but subsequently decreased and was not detected from 125 days onwards.

3.2. Long-chain alkyl diols

Four major LCDs were identified in this incubation study at the beginning of incubation, i.e. the C_{28} 1,13-, C_{30} 1,13-, C_{30} 1,15- and C_{32} 1,15-LCD. The C_{28} 1,13-LCD (Fig. 3A) showed concentrations of 0.7 ± 0.1 µg/g (E1), 1.1 ± 0.2 µg/g (E2), 0.4 ± 0.2 µg/g (E3) and 0.3 ± 0.0 µg/g (E4) and was the least abundant of the four major LCDs. The C_{30} 1,13-LCD (Fig. 3B) was 7.5 ± 4.7 µg/g (E1), 19.7 ± 4.7 µg/g (E2), 2.9 ± 1.6 µg/g (E3) and 4.4 ± 1.7 µg/g (E4), while the C_{30} 1,15-LCD (Fig. 3C) showed concentrations of 5.2 ± 2.3 µg/g (E1), 11.0 ± 2.9 µg/g (E2), 1.4 ± 0.7 µg/g (E3) and 4.2 ± 1.3 µg/g (E4). The C_{32} 1,15-LCD (Fig. 3D) was the most dominant LCD with 124 ± 61 µg/g (E1), 163 ± 47 µg/g (E2), 17.4 ± 8.7 µg/g (E3) and 55.0 ± 26.7 µg/g (E4). Additionally, a low abundance of the C_{32:1} 1,15-LCD was detected.

The concentration trends of LCDs over time developed in two phases and individual LCDs show similar concentration patterns. In the first phase, spanning the first 93 incubation days, summed LCD concentrations increased and then decreased to a minimum at 93 degradation days (Fig. 2C). Free-extractable (E1) and free- and extractable-bound (E2) LCDs decreased in concentration during the first 3 days of incubation by 42% and 52% and subsequently increased to reach maximum concentration at days 51 with 270 ± 80 µg/g for free-extractable (E1) LCDs and maximum concentration at day 22 with 330 ± 50 µg/g for free- and extractable-bound (E2) LCDs. Residually ester-bound (E3) LCDs showed the highest maximum concentrations in this first phase at day 51 with 590 ± 350 µg/g, while residually-bound glycosidic ether- or amide-bound (E4) LCDs stayed overall lower and showed highest concentration at day 35 with 200 ± 40 µg/g.

In the second phase, summed LCD concentrations reached even higher concentrations. At day 125, concentrations were substantially higher compared to initial values at day 0 of incubation time: doubled in concentration to 420 ± 190 µg/g in the E1 fraction, a fivefold increase to 1190 ± 540 µg/g in fraction E2, a thirtyfold increase to 760 ± 400 µg/g in fraction E3 and a fivefold increase to 380 ± 80 µg/g in fraction E4. After this maximum, concentrations stayed approximately constant until the end of the experiment for residually ester-bound (E3) and glycosidic ether- or amide-bound (E4) LCDs, while both free-extractable (E1) and free- and extractable-bound (E2) LCDs decreased sharply at the end of the experiment to concentrations of 230 ± 30 and 330 ± 60 µg/g respectively.

In two instances, at incubation days 51 and 69, the concentrations of LCDs in E2 were below the concentrations of E1, which is unexpected as hydrolysis should potentially result in additional release of LCDs. However, in most cases the majority of LCDs are present as free-extractable LCDs and the amount released by base and acid hydrolysis is relatively low. Possibly the lower amount of LCDs after the hydrolysis steps are due to work up losses during consecutive hydrolyses or artifact formation, i.e. the formation of polymer artifacts by condensation reactions in alkaline or acidic conditions binding LCDs to other compounds, as described in the context of algaenan isolation (Allard et al., 1997, 1998).

The LDI showed some variation over the course of the experiment, each fraction having distinct values (Fig. 4). The LDI calculated from residually-bound glycosidic ether- or amide-bound (E4) LCDs ranged from 0.34 ± 0.03 (day 10) to 0.55 ± 0.03 (day 125), free- and extractable-bound (E2) and residually ester-bound (E3) ranged from 0.31 ± 0.02 (day 15) to 0.40 ± 0.01 (day 125) and 0.28 ± 0.01 (day 22) to 0.52 ± 0.04 (day 10), respectively. The LDI calculated from free-extractable (E1) LCDs had the lowest values and ranged from 0.19 ± 0.03 (day 15) to 0.43 ± 0.25 (day 0).
The largest variability was observed during the first 35 incubation days. The LDI for the free-extractable (E1) LCDs dropped from 0.43 ± 0.25 at the beginning of the experiment to 0.25 ± 0.02 at day 3, thereafter remaining approximately constant. The LDI for the residually ester-bound (E3) LCDs showed a maximum at day 10 of 0.52 ± 0.04 and slowly dropped to constant values by day 35 of 0.32 ± 0.00, approximately remaining at that level for the remainder of the experiment, while the LDI for the residually-bound glycosidic ether- or amide-bound LCDs decreased from an initial value of 0.48 ± 0.01 to 0.34 ± 0.03 by day 10, recovering to initial values by degradation day 15 and staying constant throughout the rest of the experiment.

4. Discussion

4.1. Impact of oxygen exposure on the abundance of fatty acids and LCDs

The occurrence of C14, C16 and C18 saturated fatty acids and the C20:5 PUFA in *Nannochloropsis oculata* is consistent with earlier studies of the lipid composition of *Nannochloropsis* species (Volkman et al., 1993; Olofsson et al., 2012; Xiao et al., 2013; Mitra et al., 2015; Balzano et al., 2017). These lipids showed significant decreases in concentration when exposed to aerobic conditions during 271 days of incubation, to the point of non-detection in the free-extractable (E1) and free- and extractable-bound fractions (E2), while the residually ester-bound (E3) and glycosidic ether- or amide-bound LCDs consequently show a higher degradation rate compared to the rate of release from the algaenan, at the end of the incubation time.

At 271 degradation days, the time span of the present incubation, a substantial decrease of LCD concentrations can be observed in the free-extractable (E1) and free- and extractable-bound fractions (E2), while the residually ester-bound (E3) and glycosidic ether- or amide-bound (E4) fractions showed an increase in concentration. If a constant supply by release of LCDs from the algaenan and a simultaneous degradation of released LCDs by oxic degradation is assumed, free-extractable and free- and extractable-bound LCDs consequently show a higher degradation rate compared to the rate of release from the algaenan, at the end of the incubation time.

In a similar incubation study, set up under anaerobic conditions, complete degradation of LCDs in *Nannochloropsis salina* was also not observed (Grossi et al., 2001; Hoefs et al., 2002), but in contrast to our results,
only a transient minor increase in LCD concentrations was observed. This is possibly due to the anaerobic nature of the incubation experiment of Grossi et al. (2001), which may have led to a limited release of LCDs from the algaenan over the studied time span. A further comparison is complicated by the fact that Grossi et al. (2001) only considered LCDs that are ester-bound to the biomass and did not take into account the individual patterns of degradation of free-extractable or residually-bound glycosidic ether- or amide-bound LCDs.

The results of this incubation study potentially have implications for our understanding of the fate of LCDs in the context of organic matter settling through the water column and the subsequent burial into the sediment. On short time scales (i.e. the settling of organic matter through an oxic water column which is in the order of days to weeks), oxygen exposure is not expected to exert a substantial effect on the LCD concentrations. Only on longer time scales, (i.e. in the context of organic matter burial into the sediment which may take multiple years to decades), does oxic, as well as anoxic, degradation lead to decreasing concentrations of LCDs in the sediment, as shown previously by Hoefs et al. (2002) and Rodrigo-Gámiz et al. (2016).

Keto-ols were not detected in this study, indicating that they were not formed by oxidation of LCDs in the timespan covered by this incubation study. Possibly, the time frame of our study was too short to form these products, or the hypothesis that keto-ols are formed by oxic degradation of LCDs, is not correct.

4.2. Impact of oxic degradation on the LDI

To examine the impact of oxic degradation of LCD distributions we focused on the LDI—although we realize that the alga species used in our study is not the main producer of LCDs in the marine environment and thus LDI values cannot be converted to temperature values following Rampen et al. (2012). Despite the large changes in concentrations of LCDs, the calculated LDI showed some variability only at the initial stages of oxygen exposure, while after 35 days, no substantially changes in the LDI were observed. Values of the LDI, however, differed strongly, depending on how the respective LCDs were present in the algal biomass. The LDI calculated from free-extractable LCDs (E1) showed much lower values compared to e.g. the LDI calculated from residually-bound glycosidic ether- or amide-bound LCDs (E4) (Fig. 4). Since the values for the LDI remained distinctly different between these differently bound LCDs throughout the degradation experiment, this suggests that LCDs were not transferred between the differently bound compound pools. Furthermore, a strong preferential degradation of one diool over another was not observed. This consistent difference in LCD distributions between the differently bound compound pools is difficult to reconcile with the hypothesis that LCDs are released from the algaenan upon oxygen exposure, as it might be expected that these released LCDs would have a rather uniform distribution. Possibly, these results suggest that LCDs are bound to the algalenan in certain ratios and are also released in these ratios upon oxygen exposure.

The fact that the LDI is not substantially affected by oxic degradation is in contrast to the study of Rodrigo-Gámiz et al. (2016), which showed a preferential preservation of 1,13- over 1,15-LCDs, which consequently impacted the LDI. However, the two studies differ in time scales of oxic degradation on LCDs: while our study represents a time window of less than a year, Rodrigo-Gámiz et al. (2016) analyzed the long term (years to decades) impact of oxic exposure on LCDs during burial in sediments. Thus, short term exposure to oxygen, for instance during the settling of LCDs through an oxic water column, is unlikely to alter the LDI.

5. Conclusion

Nannochloropsis oculata biomass was aerobically degraded for 271 days. While saturated fatty acids and the C20:5 PUFA were degraded to a large extent, the concentrations of LCDs increased over time and were probably released from the biopolymer algaenan. In contrast to this release and the large changes in LCD concentration, the LDI calculated from the differently bound LCDs stayed approximately constant throughout the degradation time. This implies that on shorter timescales, the LDI is not impacted by oxygen exposure.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.orggeochem.2018.08.003.

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