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Effect of *n*-dodecane on *Crypthecodinium cohnii* fermentations and DHA production

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Abstract The potential use of *n*-dodecane as an oxygen vector for enhancement of *Crypthecodinium cohnii* growth and docosahexaenoic acid (DHA) production was studied. The volumetric fraction of oxygen vector influenced the gas–liquid volumetric mass transfer coefficient k_La positively. The k_La increased almost linearly with the increase of volumetric fraction of *n*-dodecane up to 1%. The stirring rate showed a higher influence on the k_La than the aeration rate. The effects of this hydrocarbon on *C. cohnii* growth and DHA production were then investigated. A control batch fermentation without *n*-dodecane addition (CF) and a batch fermentation where *n*-dodecane 1% (v/v) was added (DF) were carried out simultaneously under the same experimental conditions. It was found that, before 86.7 h of fermentation, the biomass concentration, the specific growth rate, the DHA, and total fatty acids (TFA) production were higher in the CF. After this fermentation time, the biomass concentration, the DHA and TFA production were higher in the DF. The highest DHA content of biomass (6.14%), DHA percentage of TFA (51%), and DHA production volumetric rate r_{DHA} (9.75 mg l^{-1} h^{-1}) were obtained at the end of the fermentation with *n*-dodecane (135.2 h). The dissolved oxygen tension (DOT) was always higher in the DF, indicating a better oxygen transfer due to the oxygen vector presence. However, since the other *C. cohnii* unsaturated fatty acids percentages did not increase with the oxygen availability increase due to the *n*-dodecane presence, a desaturase oxygen-dependent mechanism involved in the *C. cohnii* DHA biosynthesis was not considered to explain the DHA production increase. A selective extraction through the *n*-dodecane was suggested.

Keywords *Crypthecodinium cohnii* · *n*-Dodecane · k_La · DHA production and CSTR

Abbreviations CF: Control batch fermentation without *n*-dodecane · CSTR: Continuous stirred tank reactor · DF: Batch fermentation with *n*-dodecane 1% (v/v) · DHA: Docosahexaenoic acid (22:6ω3) · DOT: Dissolved oxygen tension · DPM: Dynamic pressure method · k_La: Gas–liquid volumetric mass transfer coefficient (h^{-1}) · PUFA: Polyunsaturated fatty acids · TAG: Triacylglycerols · TFA: Total fatty acids · r_{DHA}: DHA production volumetric rate (g l^{-1} h^{-1}) · rpm: Rotations per minute · r_{TFA}: TFA production volumetric rate (g l^{-1} h^{-1}) · vvm: Volume of gas per volume of aerated liquid per minute

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Introduction

Docosahexaenoic acid (DHA, 22:6ω3) is regarded to be essential for the proper visual and neurological development of infants [1–3]. Despite being an important polyunsaturated fatty acid (PUFA) in human breast milk, in the past, DHA was generally absent from infant formulas [4]. However, the World Health Organization (WHO), the British Nutritional Foundation (BNF), the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN), and the International Society for the Study of Fatty Acids and Lipids (ISSFAL) have recognized the importance of DHA and arachidonic

acid (AA) and recommended that long-chain PUFA should be included in all infant formulas [5]. Presently, over 50% of all infant formulas in the United States contain a blend of DHA and AA [6].

The traditional source of omega-3 fatty acids is fish oil. However, the use of fish oil as a food additive is limited due to problems associated with its typical fishy smell, unpleasant taste, and poor oxidative stability. Furthermore, the presence of eicosapentaenoic acid (EPA, 20:5ω3) in fish oil is undesirable for application in infant food as this fatty acid is associated with neonate growth retardation [7, 8]. Moreover, fish oil ω3 PUFA content fluctuates widely as the fish stokes are declining. Therefore, alternative sources are of interest. Microalgae biomass is particularly suitable for the extraction and purification of individual PUFA due to its stable and reliable composition. In addition, PUFA from cultured microalgae are cholesterol free, contaminant free [e.g. heavy metals, polychlorobiphenyls (PCBs)], and taste good [9]. Attempts have been made to produce DHA phototrophically by growing microalgae in photobioreactors, but it is difficult to achieve high biomass concentration and high DHA productivities. This is due to unsolved problems, namely light limitation and oxygen accumulation, in photoautotrophic cultures [10]. Screening of microalgae for heterotrophic production potential of DHA is therefore of potential significance. The heterotrophic microalga *Crypthecodinium cohnii* is an interesting source for DHA production [11–13], and for research on DHA biosynthesis [14–18], due to its unique fatty acid composition. *C. cohnii* can accumulate a high percentage of DHA [25–60% of the total fatty acids (TFA)] in its triacylglycerols (TAG) with only trivial amounts of other PUFA [19]. This characteristic makes the DHA purification process from this microorganism very attractive particularly for pharmaceutical applications, since the inclusion of PUFA as a drug component requires its purification to over 95% [20].

Important parameters for optimal DHA production include growth rate, final biomass concentration, total lipid content, and DHA proportion of the lipid. Significant lipid (TAG) accumulation does not occur during active growth in a nutrient-replete medium; it occurs during idiophase, after a culture nutrient other than the carbon source is depleted. The limiting nutrient is usually nitrogen. Therefore, industrial *C. cohnii* fermentations are usually a carbon-fed batch and progress in two stages. The first is the active growth phase during which the lipid content of the biomass is about 20%. Once the nitrogen source is depleted, carbon is continuously supplied to the fermenter. Since cell growth and division is halted due to the lack of nitrogen for de novo protein and nucleotide synthesis, the supplied carbon is converted into a storage lipid (TAG) rich in DHA [6].

The aeration conditions are of crucial importance for *C. cohnii* cells growth as the specific growth rate decreased when the cells are growing under restricted supply oxygen conditions [21]. Moreover, oxygen transfer is likely to be a limiting factor during a commercial-scale high-cell-density cultivation of *C. cohnii*, leading to the process productivity decrease. In such conditions, to maintain aerobic conditions, a very high stirrer speed had to be maintained during a large part of the process, resulting in the power input increase [22]. On the other hand, cell proliferation of dinoflagellates is negatively affected by mechanical agitation [19, 23], the way generally used to improve mass transfer in submerged fermentations.

It is possible to increase the oxygen transfer in microbial fermentations by adding an organic phase with a higher affinity for oxygen (oxygen vector) [24]. Whereas no more than the saturation concentration of oxygen can be dissolved in the aqueous phase, the supply of oxygen to the aqueous phase from the gas stream may be supplemented by equilibrium partitioning of dissolved oxygen from the organic phase to the aqueous phase. Several research groups have applied oxygen vectors to enhance oxygen supply and, as a consequence, to increase the biomass in different culture systems [10, 25, 26]. The main oxygen vectors used in biotechnology are hydrocarbons such as *n*-dodecane and *n*-hexadecane [27–32], perfluorocarbons [26, 33, 34] as well as vegetable oils [35]. In addition, biocompatible organic solvents have been used in milking microalgae products such as β-carotene from *Dunaliella salina* in two-phase bioreactors. In this process, the continuous simultaneous production and selective extraction of product from cells led to higher productivity [36].

The present work studied the impact of a hydrocarbon (*n*-dodecane) addition on *C. cohnii* cultures and DHA production. The effect of different hydrocarbon concentrations on the gas–liquid volumetric transfer coefficient k_La was previously assessed in the system.

Materials and methods

k_La measurements

A 2-l continuous stirred tank reactor glass vessel with 1.5-l working volume (SGI, France), fitted with two equally spaced baffles and equipped with one four-blade Rushton turbine, with a diameter equal to 48 mm was used. The stirrer was placed on the shaft at a distance of 5.2 mm from the vessel bottom. The sparging system consisted of a five-hole (0.5 mm diameter) single ring sparger with 6 cm diameter, placed 2.2 mm from the vessel bottom.

k_La was measured at two aeration rates (0.5 and 1 vvm) and two stirring rates (250 and 500 rpm). The dissolved oxygen concentration was measured using a polarographic probe (Mettler Toledo, Columbus, USA). It was assumed that the response of the oxygen electrode to a change in the dissolved oxygen concentration was sufficiently fast in the analyzer, since in all the runs in this work the k_La was less than 0.1 s^{-1} [37].

The data acquisition was performed using the DATA TAKER DT 500 Series 3 software (Rowville, Australia).

The gas–liquid transfer coefficient was measured by the dynamic pressure method (DPM) described by Linek et al. [38]. The principle of the method lies in the quick change of the total pressure in the vessel and the consequent recording of the oxygen probes response in liquid phase in each stage [39]. All the experiments were carried out at 27 °C.

Oxygen vector

n-Dodecane (p.a., Merck, Darmstadt, Germany; density 750 g l^{-1} at 20°C; oxygen solubility 54.9×10^{-3} g l^{-1} at 35°C and atmospheric air pressure [40]) was added to tap water in fractions of 0.5, 1, and 10% (v/v).

Crypthecodinium cohnii fermentations

Growth conditions

Crypthecodinium cohnii CCMP 316 was obtained from the Provasoli–Guillard Center for Culture of Marine Phytoplankton (CCMP) Culture Collection (Maine, USA) and was maintained in axenic conditions by subculturing every 2 weeks in f/2 + NPM medium [41–43] supplemented with glucose (6 g l^{-1}). Cultures were grown on 500 ml of the f2 + NPM medium supplemented with glucose (15 g l^{-1}) in 2-l shake flasks at 120 rpm and 27 °C in the dark. After 3 days (exponential growth phase) these cultures were used to inoculate the bioreactors with 10% (v/v) inoculum.

Bioreactor experiments

The growth medium contained 2 g l^{-1} yeast extract, 25 g l^{-1} NaCl, and 0.05% (w/v) silicone as antifoaming. The glucose (20 g l^{-1}) was sterilized separately and mixed with the other components after cooling to make up the fermentation medium. The pH value was then adjusted to 6.5 by adding a concentrate sodium hydroxide or acid solution of 1 M NaOH or 1 M HCl. The pH of the fermentation medium was measured with a Mettler Toledo steam-sterilizable pH electrode (Columbus, USA) and was controlled with a pH controller (SGI, Mountain View, USA). The dissolved oxygen tension (DOT) in the medium was measured with a Mettler Toledo oxygen electrode (Columbus, USA) and recorded in a recorder (SGI, Mountain View, USA). The stirring rate (100–600 rpm) was manually increased whenever the DOT was below 40%. Nutrient pulses (glucose and/or yeast extract) were added whenever the residual glucose concentration in the broth decreased below 10 g l^{-1} or the dissolved oxygen increased above 80%, except at the end of the fermentation. In this way, the exponential phase could be followed by a nutritional limitation other than glucose, in order to enhance lipid production [19].

The aeration rate was 1 vvm (75 l h^{-1}).

A control fermentation (CF) and a fermentation wherein an oxygen vector (*n*-dodecane, 1% v/v) was added (DF) were carried out under the same experimental conditions at the same time.

Organic phase

n-Dodecane (Merck, p.a.) was added to *C. cohnii* fermentations at a concentration of 1% (v/v).

Dry cell weight measurements

Biomass, expressed as dry cell mass, was measured gravimetrically on 5 ml culture samples, from which cells were harvested and dried for 24 h at 100°C. Biomass data resulted from the average of a minimum of two representative samples.

Glucose measurements

The residual glucose concentration in samples from the culture medium was analyzed by the 3,5-dinitrosalicylic acid method [44]. Glucose data resulted from an average of at least three representative samples.

Fatty acid analysis

Fatty acids' extraction and preparation of methyl esters were carried out according to Lepage and Roy [45] with modifications. Freeze-dried samples of *C. cohnii* (100 mg) were transmethylated with 2 ml of methanol:acetyl chloride (95:5 v/v) and 0.2 ml heptadecanoic acid (5 mg ml^{-1}, Nu-Check-Prep, Elysian, USA) as internal standard. The mixture was sealed in a light-protected Teflon-lined vial under nitrogen atmosphere and heated at 80°C for 1 h. The vial contents were then cooled, diluted with 1 ml water, and extracted with 2 ml of *n*-hexane. The hexane layer was dried over Na_2SO_4, evaporated to dryness under nitrogen atmosphere, and redissolved in hexane, which contained the methyl esters. The methyl esters were then analyzed by gas–liquid chromatography on a Varian 3800 gas–liquid chromatograph (Palo Alto, USA), equipped with a flame ionization detector. Separation was carried out on a 0.32 mm×30 m fused silica capillary column (film 0.32 μm) Supelcowax 10 (Supelco, Bellafonte, PA, USA) with helium as carrier gas at a flow rate of 3.5 ml min^{-1}. The column temperature was programmed at an initial temperature of 200°C for 8 min, then increased at 4°C min^{-1} to 240°C and held there for 8 min. Injector and detector temperatures were 250 and 280°C, respectively, and split ratio was 1:50. Peak identification was carried out using known standards (Nu-Chek-Prep, Elysian, USA). Duplicates of each sample were carried

out and each duplicate was injected twice so that the final result was obtained from an average of four values.

Results and discussion

k_La measurements

The influence of different oxygen-vector (*n*-dodecane) volumetric fractions in tap water on the gas–liquid volumetric mass transfer coefficient k_La was investigated and the results are depicted in Fig. 1. The volumetric fraction of oxygen-vector ϕ had a significant influence on k_La. For small volumetric fractions (0.5 and 1%), k_La increased almost linearly with the increase of ϕ. Above 1% (v/v), this linearity was broken. Similar results were obtained by Jia et al. [29], Jialoong et al. [30], and Rols et al. [40]. Accordingly to Rols et al. [40], this broken slope region may be due to an increase in the apparent viscosity, which might affect the oxygen transfer rate.

The k_La was also determined at two aeration rates (0.5 and 1 vvm) and two stirring rates (250 and 500 rpm). At $\phi = 10\%$, the aeration rate increase (from 0.5 to 1 vvm) brought about an increase in k_La of about 30%, whilst the stirring rate increase (250–500 rpm) led to an increase in k_La of about 40%. The same tendency was observed for the other *n*-dodecane volumetric fractions. It was clear that in general, the k_La enhancement due to the stirring rate increase was more pronounced than due to the aeration rate increase. These results are in accordance to Doran [46] who stated that the stirring rate increase has a higher influence on the k_La than the aeration rate.

The advantage of using an oxygen vector in microbial cultures is that it increases the oxygen transfer rate from the gas phase to the micro-organisms without the need for extra energy supply and the consequent shear stress increase which may affect sensitive organisms such as microalgae [23]. Oxygen vectors can act as surface-active agents to lower the surface tension of water and increase the gaseous specific interfacial area [4].

Crypthecodinium cohnii fermentations

Previous work (not published) demonstrated that the addition of *n*-dodecane as an oxygen vector up to volumetric fractions of 1% to the microalga *C. cohnii* shake flask cultures increased the biomass yield. However, at *n*-dodecane volumetric fractions higher than 1%, *C. cohnii* cell lysis was observed. For that reason, this hydrocarbon was added to *C. cohnii* fermentations at the volumetric fraction of 1%.

The effect of the *n*-dodecane presence on *C. cohnii* batch cultures is shown in Fig. 2. The stirring rate changes during the fermentation time course are depicted in Fig. 2a.

The biomass concentration measured in CF seemed to be higher than that in the DF, until 86 h. The specific growth rate, calculated in this time period (24–86 h), was 0.020 h^{-1} for the CF and 0.016 h^{-1} for the DF.

At 68.5 h, a pulse of nutrients (glucose and yeast extract) was added as the DOT attained 100 and 80% for the DF and CF, respectively, indicating that a nutrient other than glucose was limiting the cell growth. At 86.7 h, the CF biomass concentration attained 14.5 g l^{-1}, whilst the biomass concentration of the DF attained 12 g l^{-1}.

At this fermentation time, a new yeast extract pulse was added as the DOT readings increased again (100 and 91.1% for CF and DF, respectively). After 86.7 h, the measured biomass concentration in DF steadily increased until the end of the fermentation (21.45 g l^{-1} at $t = 135.2$ h), whilst the CF biomass attained lower

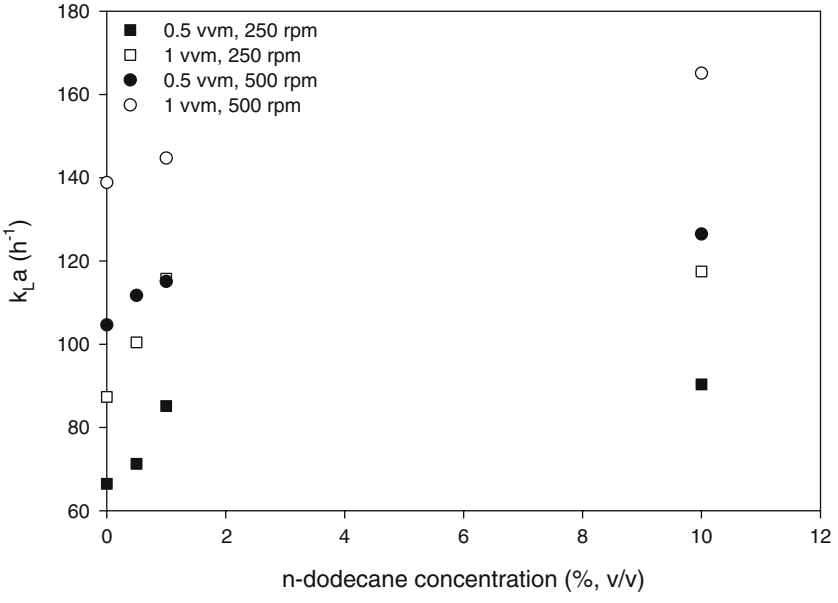

Fig. 1 Effect of *n*-dodecane volumetric fractions on the gas–liquid volumetric transfer coefficient k_La for different stirring rates and aeration rates

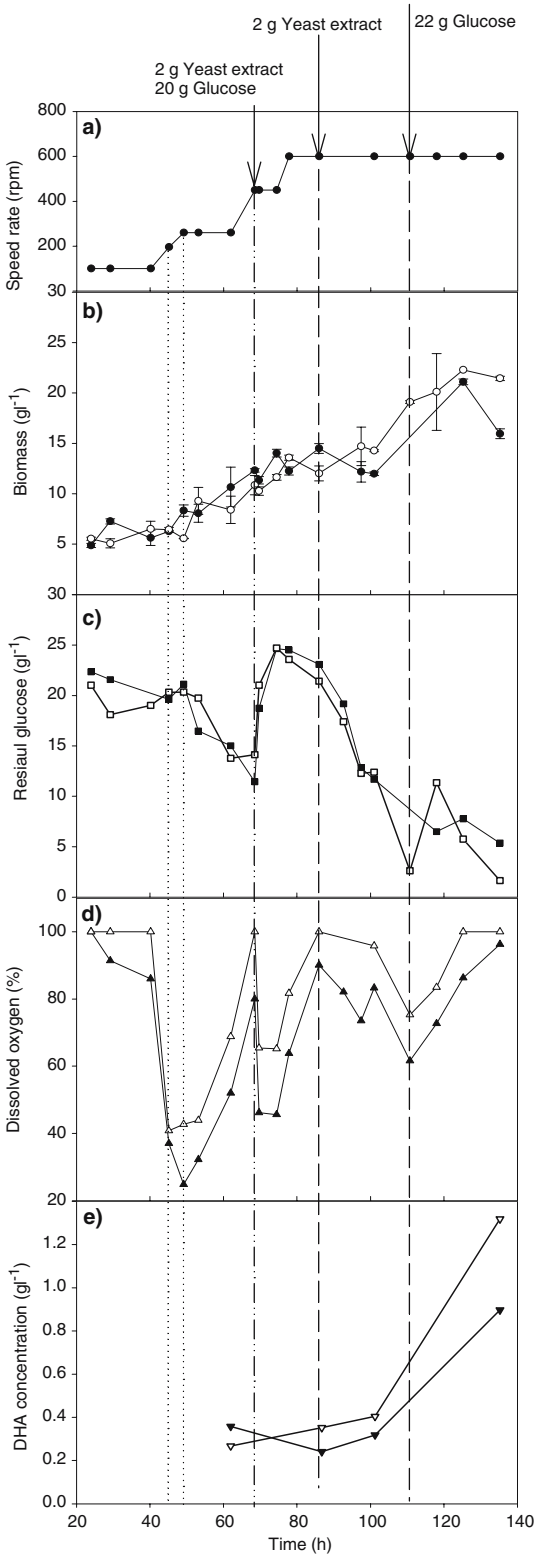

Fig. 2 Effect of *n*-dodecane addition on *C. cohnii* batch profile fermentation. *Filled symbols* control fermentation, *open symbols* fermentation with *n*-dodecane 1% (v/v), *dotted lines* speed rate increase, *dash lines* nutrient addition, *dash-dotted lines* speed rate increase and nutrient addition. (**a**) speed rate, (**b**) biomass, (**c**) dissolved oxygen, (**d**) DHA concentration

biomass concentration (15.95 g l^{-1} at $t = 135.2$ h). It seemed that, after an adaptation period to the *n*-dodecane presence (86.7 h), wherein the cell growth was affected, the microalga recovered and attained higher dry cell weight values. This is supported in Fig. 2c, where it is shown that after 77.8 h, *C. cohnii* glucose uptake in the presence of *n*-dodecane was higher than the glucose uptake in the absence of *n*-dodecane, in most of the time points measured.

At 110.7 h, after an active cell growth phase, a new pulse of glucose was added, in order to achieve the nutrient requirements for lipids accumulation. It is known that after the exponential phase, when a nutrient is depleted (preferably the nitrogen), the carbon source must be supplied to the cells, so that the supplied carbon is converted into storage lipids which are rich in DHA in *C. cohnii* cells [6].

Significant differences were found between the DOT profiles of both fermentations (Fig. 2d). The DOT readings in the presence of *n*-dodecane were always higher than those observed in the absence of the organic solvent (1.7 times higher at $t = 49$ h) due to the presence of the oxygen vector. Even when the biomass concentration measured in DF was higher than that in CF ($t > 86.7$ h), the DOT in the former fermentation remained higher indicating an enhanced mass transfer through the oxygen-vector addition. A similar behavior was reported by Rols et al. [40] who used *n*-dodecane as an oxygen vector at different volumetric fractions to study the effect of this hydrocarbon on *Aerobacter aerogenes* cultures.

The effect of the *n*-dodecane presence on *C. cohnii* DHA production is shown in Fig. 2e. At 62 h, cells growing in the absence of *n*-dodecane produced more DHA (0.36 g l^{-1}) than cells growing in the presence of the hydrocarbon (0.27 g l^{-1}). However, this tendency was inverted at 86.7 h, when the DHA produced by *C. cohnii* cells growing in the presence of the *n*-dodecane increased until the end of the fermentation, attaining 1.32 g l^{-1} at 135.2 h. During the same time period, the DHA produced by the cells growing in the *n*-dodecane absence reached a minimum at 86.7 h, and afterwards increased until the end of the fermentation, attaining 0.90 g l^{-1} at 135.2 h.

The DHA content of the biomass, the DHA percentage of TFA, and the DHA production volumetric rate (r_{DHA}) followed a similar pattern (Fig. 3a–c). After a minimum at 86.7–101 h, *C. cohnii* cells increased their DHA content, concentration, and productivity until the end of the fermentation (135.2 h). The DHA content in biomass, concentration, and productivity minimum observed at 86.7 and 101 h might be due to the high yeast extract concentration in the broth, resulting from the previous addition. As already mentioned, the DHA production is enhanced when the nutrient limitation occurs, especially nitrogen. As the yeast extract is the nitrogen source, its presence in the broth might have

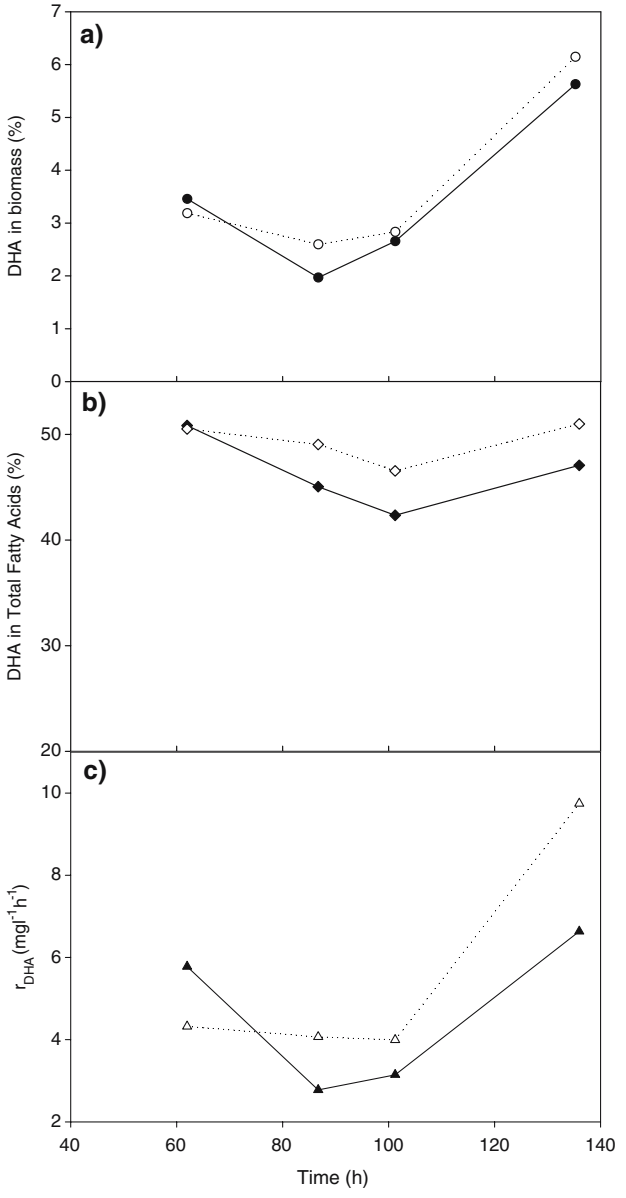

Fig. 3 Effect of *n*-dodecane addition on *C. cohnii* DHA content of biomass (**a**), DHA percentage of total fatty acids (**b**) and DHA productivity (**c**). *Filled symbols* control fermentation, *open symbols* fermentation with *n*-dodecane 1% (v)

decreased the DHA production/productivity. In fact, it has been reported that the lipid content of *C. cohnii* cells decreased markedly at high yeast extract concentrations [47].

From 86.7 h until the end of the fermentation, the DHA content in biomass, DHA percentage of TFA, and r_{DHA} of the DF was higher than those measured in the CF. The DHA content of biomass produced in the *n*-dodecane presence attained 6.14 g l^{-1} at 135.2 h, 9% more than the DHA produced in the absence of the organic solvent. The DHA percentage in the TFA in the presence of the *n*-dodecane was 51%, 9% more than the DHA percentage in the TFA found in *C. cohnii* cells grown in the *n*-dodecane absence. The r_{DHA}, a crucial parameter in DHA production by microbial fermentation [48], attained 9.75 mg l^{-1} h^{-1} in the DF, 47% more than the r_{DHA} obtained in the CF.

De Swaaf et al. [47] calculated the amount of lipid from the total amount of fatty acids and the internal standard. As observed for the DHA content, concentration, and productivity; the TFA percentage of biomass, the TFA production, and the TFA volumetric rate increased at the end of the fermentation (Fig. 4).

Although the TFA content of cells grown in the absence and in the presence of *n*-dodecane was almost the same (≃12% at 135.2 h, Fig. 4a), the DHA percentage of the TFA was higher in the later situation (Fig. 3b).

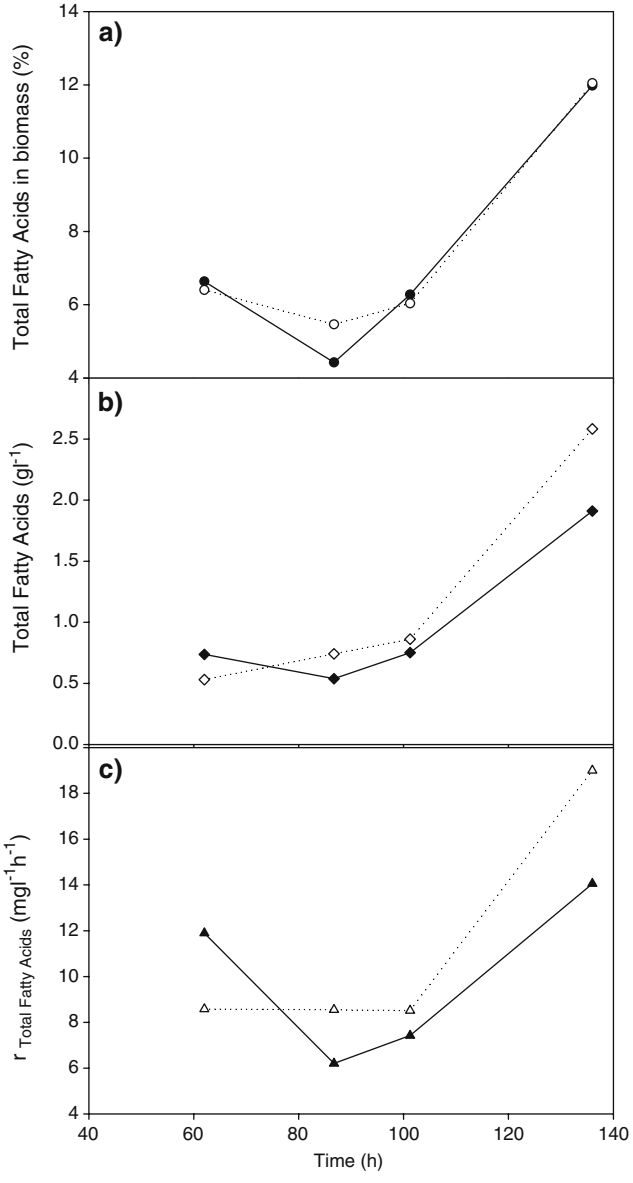

Fig. 4 Effect of *n*-dodecane addition on *C. cohnii* TFA content of biomass (**a**), TFA concentration (**b**) and TFA productivity (**c**). *Filled symbols* control fermentation, *open symbols* fermentation with *n*-dodecane 1% (v)

The TFA concentration generally increased with the time fermentation and was higher in the DF, reaching 2.58 g l^{-1} at the end of fermentation (35% more than the TFA concentration in the CF) (Fig. 4b). After 86.7 h, the r_{TFA} was also higher in the DF, attaining 19 mg l^{-1} h^{-1} at the end of the fermentation, 35% more than the r_{TFA} obtained in the CF (Fig. 4c).

Table 1 shows *C. cohnii* fatty acid composition analyzed in cells taken at different fermentation times. The total unsaturated fatty acids was higher in the cells growing in the presence of *n*-dodecane, which was mainly due to the DHA increase, as the other fatty acids remained almost unchanged. Several factors might have contributed for this finding.

It has not been cleared whether desaturases are involved in the *C. cohnii* DHA production [18, 49]. It has been suggested that the final step leading to the DHA production is distinct from that in most of the microorganisms, where the saturated fatty acids are successively desaturated and elongated through a series of reactions, using the fatty acid synthetase (FAS) complex of enzymes, leading to the formation of various PUFAs [18, 50]. In fact, Sonnenborn and Kunay [51] reported in vitro the production of saturated fatty acids, mainly 14:0 and 16:0, by a purified cytosol enzyme complex of *C. cohnii*. They concluded that the FAS system should be operative in *C. cohnii* and might supply the precursors for the DHA biosynthesis. However, Beach et al. [14] incubating *C. cohnii* cells in the presence of ^{14}C-labeled fatty acids ranging from 10 to 18 carbon atoms found that label was detected in oleic acid (18:1, the most abundant monounsaturated fatty acid in *C. cohnii*) but not in DHA. De Swaaf et al. [49] reported that de novo synthesis of DHA with desaturases might occur, although it has not been cleared whether oxygen-dependent desaturases are active in the biosynthesis of DHA in *C. cohnii*. However, the system appeared to be aerobic as, in a study of Beach and Holz [21], the DHA and monounsaturated fatty acids sharply decreased when a growing culture of *C. cohnii* was switched from gassing with air to nitrogen gas. In the present work, it was demonstrated that the oxygen vector presence allowed a higher oxygen availability in *C. cohnii* fermentations, and in this case, the DHA productivity increased, but the other unsaturated fatty acids remained almost unchanged. This fact suggests that probably another factor led to *C. cohnii* DHA production increase in the presence of the hydrocarbon.

Biocompatible organic solvents have been used in milking microalgae products such as β-carotene from *D. salina* in two-phase bioreactors, leading to higher productivity [36]. In the present work, the use of *n*-dodecane might have worked as a selective extraction solvent, removing TAG which, in *C. cohnii* cells, are known to be rich in DHA. It has been reported that storage lipids (TAG) are easier extracted than structural lipids when using nonpolar organic solvents [52]. As storage lipids (TAG) have protective effect on the cells, allowing their survival during starvation periods, its extraction will stimulate their substitution by newly synthesized lipids, thereby enhancing its production. In this way, the TFA produced by *C. cohnii* cells grown in the *n*-dodecane presence would be enriched in DHA. This mechanism could explain the higher DHA productivity observed in *C. cohnii* cells grown in the presence of *n*-dodecane although the TFA content in *C. cohnii* cells grown in the presence and in the absence of *n*-dodecane was nearly the same, as mentioned above.

Unfortunately, the low *n*-dodecane volumetric fraction used in this work did not allow the extracted fatty acid analysis in the organic phase, as no hydrocarbon was found in the broth at the end of the fermentation. Two factors might have contributed for this observation. First, the high stirring rate used after 77.8 h (600 rpm) might have broken the *n*-dodecane droplets into very small droplets so that an emulsion *n*-dodecane-aqueous broth could have formed, hindering the organic phase separation from the aqueous phase. Second, it has been reported that *n*-dodecane is absorbed by *D. salina* cells specially in the cell membrane [36]. The same mechanism could have occurred in our system.

Conclusions

In this work, it was possible to increase the DHA production by *C. cohnii* cells through the addition of *n*-dodecane, a cheap solvent which can be used simultaneously as an oxygen vector, enhancing the oxygen transfer, and as an organic phase, with the ability to

Table 1 Influence of *n*-dodecane presence on *C. cohnii* cellular fatty acid composition at different fermentation times

Fermentation time (h)	12:0	14:0	16:0	16:1ω9	18:0	18:1ω9	22:5ω3	22:6ω3	% Sat.	% Unsat.
CF										
62.0	2.0±0.0	11.6±1.7	23.6±0.6	0.2±0.0	5.0±0.8	8.6±1.3	0.4±0.1	50.8±1.0	42.3	60.1
86.7	4.0±1.1	11.7±0.8	25.2±1.6	0.3±0.2	3.9±2.2	9.3±2.1	0.5±0.3	45.0±5.4	44.8	55.0
101.2	2.1±0.4	10.3±0.9	25.3±0.2	0.5±0.1	7.0±0.3	11.6±0.8	0.9±0.2	42.3±0.7	44.8	55.4
136.0	7.3±0.7	15.6±0.3	20.6±0.3	0.2±0.0	2.3±0.3	6.6±0.2	0.3±0.2	47.1±1.2	45.8	54.2
DF										
62.0	1.3±0.1	9.5±0.9	24.3±0.4	0.2±0.1	4.3±1.6	9.4±1.5	0.5±0.6	50.5±2.7	39.4	60.6
86.7	2.3±0..3	10.7±1.6	24.1±1.5	0.3±0.1	3.6±2.0	9.7±1.9	0.3±0.2	49.0±6.8	40.7	59.0
101.2	2.3±1.1	10.0±1.6	23.9±1.0	0.4±0.1	5.1±0.7	10.8±0.7	0.9±0.2	46.5±3.2	41.3	58.7
136.0	4.7±0.9	12.3±1.6	20.7±0.6	0.2±0.0	2.7±0.9	8.2±0.6	0.3±0.2	51.0±1.5	40.4	59.6

increase the DHA production and to concentrate this fatty acid, which will reduce the downstream purification costs. These conditions seem to be suitable for the large-scale DHA production from *C. cohnii*.

A new strategy to improve the DHA production from this microalgae in two-phase large-scale bioreactors is now in progress.

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