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Disruption of microalgal cells for lipid extraction through Fenton reaction: Modeling of experiments and remarks on its effect on lipids composition

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HIGHLIGHTS

- A mathematical model of cell disruption through Fenton reaction is proposed.
- Model results well capture experimental data in terms of extracted lipids.
- An explanation of the effect of disruption on final FAMES composition is proposed.
- Optimization maps are shown and sensitivity analysis is performed.
- The model might be exploited to viably apply the technique at the industrial scale.

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ABSTRACT

A novel cell disruption technique, based on the use of Fenton reaction, for the improvement of lipid extraction from microalgae has been recently proposed in the literature. The experimental results have shown that, when disruption is performed under suitable operating conditions, the amount of lipids subsequently extracted from *Chlorella vulgaris* is greatly increased with respect to the case where no pre-treatment was performed. Specifically, the use of Fenton reactant leads to a corresponding increase of the extracted lipids from 6.9 to 17.4 %wt/wt. Moreover, it is observed that the treatment provokes a significant improvement of the quality of fatty acids methyl esters (FAMEs) obtained by trans-esterification of extracted lipids. These results, together with the extreme simplicity, low cost and energy consumption of the proposed technique, are very promising in view of its industrial transposition. To this aim, the use of suitable mathematical models might represent a valuable tool to design and control possible industrial size reactors. Along these lines, a simple but exhaustive model to quantitatively describe the effects of contact time and reactants concentration on the amount of lipids extractable from microalgae is proposed in this work. The model takes into account the effect of the $OH^{\cdot}$ species on both cell wall breakage and lipid peroxidation phenomena. Model results and experimental data are successfully compared in terms of lipids extracted from wet microalgae previously subjected to the disruption treatment under different operating conditions. Furthermore, the possible chemical physical mechanisms underlying the improvement of FAMES composition, observed after the disruption treatment, is discussed. Finally, potential capabilities of the model to contribute to the industrial scale transposition of the proposed technique are presented.

1. Introduction

It is well recognized that microalgae represent today one of the most promising feedstock for the production of biofuels in a biorefinery framework which might involve the capture of CO_2 from flue gases and the co-production of high added value chemicals [1–3]. However, in order to be viably implemented at the industrial scale, the microalgae based technology should be

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properly optimized in terms of reduction of the operating costs deriving from the different unit steps of the process [4].

In particular, the development of effective and sustainable processes for the extraction of lipids from microalgae cells is crucial in facilitating the industrial-scale production of microalgal biodiesel [5]. In fact, lipids used for the production of the latter one are contained as droplets within the microalgae cytoplasm confined by a rigid cell wall, and thus have to be extracted in order to be suitably exploited. The extraction of algal lipids can be performed starting either from wet or dry microalgal biomass. However, since drying of microalgal biomass is prohibitive from the energy and economic point of view, lipid extraction from wet biomass is generally preferred for industrial applications [5,6]. On the other hand, most of lipid extraction technologies are based on the use of solvents which can be either of organic nature or represented by supercritical fluids. However, when solvent extraction is applied to wet biomass, the microalgal cells tend to remain in the water phase due to their surface charges and are not going to contact the solvent phase which allows lipid extraction [7]. For this reason, in order to apply the solvent extraction technique directly on the wet biomass, the latter one should be subjected to a pre-treatment aimed to break the cell wall, thus permitting the subsequent release of intracellular lipids into the liquid bulk. This way, when the disrupted biomass is contacted with solvents, lipids released from the algal cell are transferred to the solvent phase due to their hydrophobicity and can be later collected by evaporating the solvent.

Several cell disruption techniques, based on chemical or physical methods, have been so far proposed in the literature.

The physical techniques include high pressure homogenization, ball milling, microwaving, ultra-sonication, electrocoagulation and hydrodynamic cavitation as well as thermolysis, osmotic shocks, laser treatments and electroporation [7–14]. Nevertheless, most of these methods are very difficult to scale up and might involve high energy consumption which might range from 33 MJ kg^{-1} to 529 MJ kg^{-1} [14]. As a result, it is apparent that the adoption of physical disruption methods would lead to an energetic imbalance which in turn might strongly affect the economic sustainability of the microalgal technology [9].

On the other hand, chemical methods for cell disruption rely on selective interaction of certain chemicals with the components of cell wall and are basically represented by enzymatic hydrolysis or chemical lyses through NaOH, HCl, H_2SO_4, HNO_2, lysine acetone, methanol and dimethyl sulfoxide [15–17]. When compared with physical methods, the chemical ones are less energy consuming while frequently showing higher disruption yields and furthermore, are simpler to scale-up. Nevertheless, even these methods show some significant drawbacks. In particular, expensive chemicals should be continuously supplied and this aspect might undermine the economic sustainability of the technology. Furthermore, acids and alkalis might attack the valuable products (i.e. lipids) of the microalgal cell, thus thwarting the whole process [7]. Therefore, novel techniques are needed to perform cell disruption by means of chemical methods.

Along these lines, a novel cell disruption technique, based on the use of Fenton reactants, for the enhancement of the lipid extraction yields from microalgae has been recently proposed by Steriti et al. [18]. Briefly, the method consists of contacting the wet biomass with a disruption solution containing suitable concentrations of $FeSO_4$ and H_2O_2, namely the Fenton reactants, for suitable periods of time. According to Steriti et al. [18], once in solution, H_2O_2 can react with the Fe^{2+} ions thus initiating the so called Fenton's radical reaction chain which leads to the production of the reactive oxygen species (ROS) $OH^{\cdot}$ [19]. The latter one, given its high reactivity, is capable to attack and effectively degrade the organic compounds constituting the microalgae cell wall, i.e. polysaccharides, cellulose, glycoproteins, phospholipids etc., thus leading to its rupture and the subsequent release of intracellular lipids in solution. It should be highlighted here that the phospholipids in the cell wall of microalgae are not useful to produce biofuels. In fact, the only category of lipids useful for producing biofuels is represented by the neutral ones which can be found as triglyceride's droplets floating in the cell cytoplasm. The experimental results obtained by Steriti et al. [18] have shown that, when disruption was performed under suitable operating conditions, the amount of lipids subsequently extracted from *Chlorella vulgaris* was more than doubled with respect to the case where a classical approach was adopted. Moreover, it was observed that, in view of the exploitation for producing biodiesel, the disruption treatment resulted in a significant improvement of the quality of FAMEs (fatty acid methyl esters) obtained by trans-esterification of extracted lipids. In fact, a great reduction of the relative content of polyunsaturated fatty acids was observed with respect to the case where no disruption treatment was performed. These results, together with the extreme simplicity of the proposed technique as well as the low cost of employed reactants and the modest energy consumptions, are very promising in view of the industrial transposition of the proposed technique.

However, the results obtained by Steriti et al. [18], have also shown that the yield of the proposed technique was quite sensitive to the specific operating conditions adopted during cell disruption. In particular, a relatively narrow range of optimal operating conditions, in terms of extracted lipids, has been identified. This result depends on the fact that, if contact time and reactants' concentration are selected at the corresponding small values, the cell wall disruption cannot be effectively completed while, if their values are set at the large side, the useful algal lipids released in solution can be degraded by the excess of $OH^{\cdot}$ radicals produced by the Fenton reaction. As a result, the optimal set of operating parameters should be identified and finely tuned when considering a possible implementation of the proposed technique on the industrial scale. To this aim, while the experimental activity allows investigating only a limited range of operating conditions, the use of suitable mathematical models might represent a suitable tool to predict the response in terms of extracted lipids of the investigated system to changes of the operating parameters in a wide range of values. Consequently, the goal of the present work is to propose a simple but exhaustive mathematical model to quantitatively describe the effects of the variation of the above operating parameters, i.e. contact time and concentration of disruption reactants, on the amount of lipids extractable from microalgae.

The proposed model takes into account the effect of radical oxygen specie $OH^{\cdot}$ produced by the Fenton reaction on both cell breakage and lipid peroxidation phenomena, thus allowing to identify the operating conditions at which the optimal compromise between these two counteracting mechanisms is achieved. It should be noted that, while several kinetic models for the simulation of the oxidation of a wide range of organic pollutants through Fenton process have been so far proposed in the literature [19–23], to the best of our knowledge, the effect of Fenton reaction on algal cell disruption has not been yet the subject of experimental or modelling investigation.

Model results and experimental data by Steriti et al. [18] were compared in terms of lipids extracted from wet biomass subjected to the proposed cell disruption treatment performed under different operating conditions. Furthermore, a possible explanation about the chemical physical mechanisms underlying the improvement of FAMES composition observed as a result of the disruption treatment is proposed.

2. Materials and methods

A brief description of the experimental procedures is reported in what follows for the sake of clarity. However, for a detailed description of materials and methods adopted during the experimental trials, the interested reader should refer to the paper by Steriti et al. [18].

2.1. Microorganism and culture conditions

A fresh water unialgal strain *C. vulgaris* was taken into account. Microalgae were grown in a modified Kolkwitz growth medium [2], under continuous flux of 100% (v/v) CO_2 within a 6 L helical tubular photobioreactor (BIOCOIL) coupled with a degasser system as described in the literature [1,18]. A light intensity of 100 $\mu E\ m^{-2}\ s^{-1}$ for a light-dark photoperiod of 12 h was provided by suitable fluorescent lamps. Once the culture reached the stationary growth phase the photobioreactor was operated in fed-batch mode. The withdrawals made during the operation in fed-batch mode were used for the cell disruption experiments. The evolution of biomass concentration C_b ($g_{dw}\ L^{-1}$) during growth was calculated from optical density (OD) measurements using a suitable C_b vs. OD calibration curve which was obtained as reported by Steriti et al. [18].

2.2. Cell disruption

Once the culture in the photobioreactor reached the stationary growth phase, microalgae were first harvested and then centrifuged to obtain a concentrated pellet of wet biomass. The exact weight of dry biomass contained in the wet pellets was evaluated by means of a suitable calibration line obtained by gravimetrically evaluating the wet weight of biomass obtained after centrifugation and its corresponding dry weight after drying at 105 °C for 24 h [18]. Next, wet pellets containing known amounts of dry biomass were subjected to the cell disruption procedure which consisted of contacting them with selected volumes of the disrupting solution within a falcon flask that was then sealed and continuously shaken at 300 rpm for certain periods of time at room temperature. The disrupting medium consisted of an aqueous solution of H_2O_2 and $FeSO_4$, i.e. the Fenton reactant. A number of experiments were performed by varying the H_2O_2 concentration in the range between zero to 6 mol L^{-1}, while keeping constant the concentration of $FeSO_4$ in the disruption solution at 0.025 mol L^{-1}. Moreover, contact times ranging from 0 to 5 min were considered [18]. For the sake of reproducibility, each experimental condition was repeated at least twice. Once the desired contact time was elapsed, the disruption reaction was suddenly stopped by diluting the entire reacting mixture ten times of its original volume through the addition of ethanol so that the concentration of disruption reactants, and thus the reaction rate, were dramatically lowered up to values very close to zero [18].

2.3. Lipid extraction and fatty acid methyl esters analysis

Neutral lipid extraction was performed directly on the wet disrupted biomass through ethanol and hexane according to a method consisting of a slight modification of the one proposed by [24]. It should be noted that, according to Steriti et al. [18], the technique consists of a first extractive step where ethanol is added to the disruption solution to promote the transfer of the products of disruption reaction, as well as of the liberated lipids, in the resulting hydroalcoholic solution. Subsequently, hexane is added in order to promote the transfer of only hydrophobic lipids from the hydroalcoholic phase to the hexanic one from which lipids can be collected by evaporating the solvent. Therefore, by using hexane, neutral lipids can be separated from all the other compounds resulting from disruption, which, due to their hydrophilicity remain in the hydroalcoholic solution. In this regard, it is important to remark here that lipid peroxides, obtained as a result of the lipid peroxidation processes triggered by the Fenton reaction (cf. Table 2), are hydrophilic compounds and thus tend to remain in the hydroalcoholic solution without being transferred to the hexanic phase. For this reason, the lipids collected by evaporating the hexanic phase are only the "non peroxidized" ones, i.e. those ones useful for producing biofuels. The percent weight of lipids extracted from the dry biomass was then obtained as the ratio between the weight of lipid obtained and the original dry weight of microalgae which was subjected to the extraction process. The fatty acid methyl esters (FAMEs) composition of extracted lipids was determined according to the European regulation/commission regulation EEC n° 2568 [25] after transesterification with methanol-acetyl chloride. To this aim a suitable chromatograph equipped with a flame ionization detector (FID) (Thermo Trace Ultra, GC-14B) and a RTX-WAX column T (fused silica, 0.25 mm × 60 m × 0.25 μm) maintained at 180 °C was used.

3. Mathematical modeling of the cell disruption with Fenton reactant

It is well known that the addition of H_2O_2 to aqueous solutions containing Fe^{2+} ions can trigger the so called Fenton's reaction which in turn involves the key reactive steps reported in Table 1 [19]. As it can be observed, the reaction steps lead to the production of free radicals such as $OH^{\cdot}$ and $HO_2^{\cdot}$ which, because of their high instability and reactivity, are capable to oxidize the organic compounds in solution, thus provoking their degradation. For this reason, the Fenton's reaction is typically exploited to remove a wide range of organic contaminants from aqueous matrices [21]. According to the same mechanism, the $OH^{\cdot}$ radicals produced by the Fenton's reactive chain may react with several organic compounds constituting the cell wall of algae, thus leading to their degradation and the consequent disruption of the protective cell wall followed by the release of intracellular lipids, as schematically shown in Fig. 1. Different constituents of the cell wall, such as for example cellulose, peptidoglycanes, xylanes and, in general polysaccharides etc., can be involved in a number of complicated reactions with $OH^{\cdot}$ radicals.

While the rigorous modeling of such phenomena would require the detailed knowledge of the intricate network of the reactive steps involved in the disruption process, in this work, a suitable lumped reaction scheme has been adopted to describe the overall disruption process. Specifically the first step consists of the following reaction:

Table 1
Steps and constant rates of the Fenton reaction [19].

ID	Reaction	Rate constant	Value	Units
	Chain initiation			
R1	$Fe^{2+} + H_2O_2 \xrightarrow{k_1} Fe^{3+} + OH^{\cdot} + OH^-$	k_1	70	$L\ mol^{-1}\ s^{-1}$
	Chain propagation			
R2	$OH^{\cdot} + H_2O_2 \xrightarrow{k_2} H_2O + HO_2^{\cdot}$	k_2	3.3×10^7	$L\ mol^{-1}\ s^{-1}$
R3	$H_2O_2 + Fe^{3+} \xrightarrow{k_3} Fe^{2+} + HO_2^{\cdot} + H^+$	k_3	0.01	$L\ mol^{-1}\ s^{-1}$
R4	$Fe^{3+} + HO_2^{\cdot} \xrightarrow{k_4} Fe^{2+} + O_2 + H^+$	k_4	1.2×10^6	$L\ mol^{-1}\ s^{-1}$
	Chain termination			
R5	$Fe^{2+} + HO_2^{\cdot} \xrightarrow{k_5} Fe^{3+} + HO_2^-$	k_5	1.3×10^6	$L\ mol^{-1}\ s^{-1}$
R6	$OH^{\cdot} + Fe^{2+} \xrightarrow{k_6} Fe^{3+} + OH^-$	k_6	3.2×10^8	$L\ mol^{-1}\ s^{-1}$

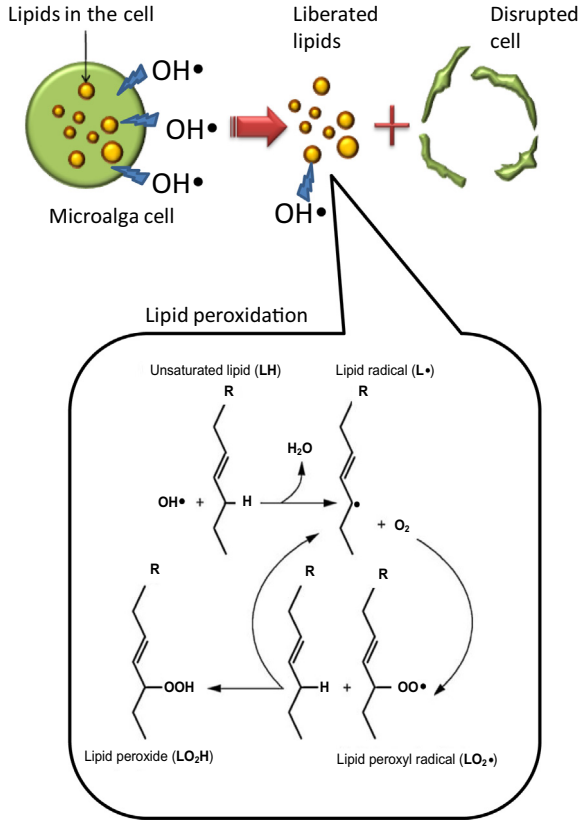

Fig. 1. Schematic representation of the cell disruption process and lipid peroxidation reactive chain adapted from [27].

$$y_{OH/X}OH^{\cdot} + X \xrightarrow{k_d} y_{L/X}LH + DX$$

The simplified reaction above states that the OH· radicals react with the algal cells (X) with constant rate k_d to produce disrupted cells (DX), and generic lipids (LH), respectively (cf. Fig. 1). The yield $y_{OH/X}$ takes into account that several molecules of OH· might be needed to produce the rupture of one mole of algal biomass [26]. On the other hand, the coefficient $y_{L/X}$ represents the moles of lipid released in the bulk for each mole of microalgae cells being disrupted. Since it can be reasonably assumed that all the lipids originally contained within the microalgal cells are released after cell disruption, the coefficient $y_{L/X}$ can be suitably evaluated starting from the actual weight percentage content of intracellular lipids of microalgae.

Once released in the liquid bulk, lipids (LH) can react with OH· radicals to initiate a reactive chain which finally leads to the production of lipid peroxides (LO_2H), water and other degradation products [27]. It is well known [28] that the initiation step of lipid peroxidation consists of the abstraction of one weakly bonded hydrogen atom from the unsaturated lipid (LH) by a free radical OH· which leads to the conversion of the lipid molecule into a lipid radical (L·) as shown in the following reaction and in Fig. 1:

$$OH^{\cdot} + LH \xrightarrow{k_{L1}} L^{\cdot} + H_2O$$

It should be noted that, according to the literature [27,28], the polyunsaturated ones are the category of lipids most susceptible of peroxidation since they contain weakly bonded hydrogen atoms, between carbon–carbon double bonds, which can react with the OH· radicals to form water (cf. Fig. 1). Once triggered, the radical reaction chain proceeds according to the steps of propagation, and termination shown in Table 2 where the corresponding reaction constants are also reported. Specifically, in the propagation phase of lipid peroxidation, the lipid radical quickly reacts with the dissolved oxygen O_2 to form a lipid peroxyl radical ($LO_2^{\cdot}$) which in turn can react with the lipids (LH) to produce a lipid hydroperoxide (LO_2H) and a new L·, as shown in Fig. 1. Subsequently, termination of lipid peroxidation takes place when lipid radical species react with each other by forming non-radical species (NRS), as shown in Table 2.

Table 2
Propagation and termination steps of the lipid peroxidation reactive chain and corresponding rate constants [29].

ID	Reaction	Rate constant	Value	Units
	Chain propagation			
R7	$L^{\cdot} + O_2 \xrightarrow{k_{L2}} LO_2^{\cdot}$	k_{L2}	$3.0 - 4.6 \times 10^8$	L mol^{-1} s^{-1}
R8	$LO_2^{\cdot} + LH \xrightarrow{k_{L3}} LO_2H + L^{\cdot}$	k_{L3}	1.9×10^1	L mol^{-1} s^{-1}
	Chain termination[*]			
R9	$L^{\cdot} + L^{\cdot} \xrightarrow{k_{L4}} NRS_1$	k_{L4}	6.6×10^4	L mol^{-1} s^{-1}
R10	$L^{\cdot} + LO_2^{\cdot} \xrightarrow{k_{L5}} NRS_2$	k_{L5}	1.0×10^5	L mol^{-1} s^{-1}
R11	$LO_2^{\cdot} + LO_2^{\cdot} \xrightarrow{k_{L5}} NRS_3$	k_{L6}	6.6×10^4	L mol^{-1} s^{-1}

[*] NRS_i = non radical species.

According to the approach typically adopted in the literature, the algal disruption and lipid peroxidation reactions above are typically considered to be governed by second order kinetics [19,27–29] and thus the following material balances can be written to quantitatively describe the time evolution of algae, lipids and lipid radicals concentration in the bulk liquid:

$$\frac{d[X]}{dt} = -k_d[OH^{\cdot}][X] \quad (1)$$

$$\frac{d[LH]}{dt} = y_{L/X}k_d[OH^{\cdot}][X] - k_{L1}[OH^{\cdot}][LH] - k_{L3}[LO_2^{\cdot}][LH] \quad (2)$$

$$\frac{d[L^{\cdot}]}{dt} = k_{L1}[OH^{\cdot}][LH] - k_{L2}[L^{\cdot}][O_2] + k_{L3}[LO_2^{\cdot}][LH] - k_{L4}[L^{\cdot}]^2 - k_{L5}[L^{\cdot}][LO_2^{\cdot}] \quad (3)$$

$$\frac{d[LO_2^{\cdot}]}{dt} = k_{L2}[L^{\cdot}][O_2] - k_{L3}[LO_2^{\cdot}][LH] - k_{L5}[L^{\cdot}][LO_2^{\cdot}] - k_{L6}[LO_2^{\cdot}]^2 \quad (4)$$

$$\frac{d[O_2]}{dt} = -k_{L2}[L^{\cdot}][O_2] \quad (5)$$

along with the following initial conditions:

$$[X] = [X^0] = X^0/MW_X \quad \text{at } t = 0 \quad (6)$$

$$[LH] = [LH]^0 = 0 \quad \text{at } t = 0 \quad (7)$$

$$[L^{\cdot}] = [L^{\cdot}]^0 = 0 \quad \text{at } t = 0 \quad (8)$$

$$[LO_2^{\cdot}] = [LO_2^{\cdot}]^0 = 0 \quad \text{at } t = 0 \quad (9)$$

$$[O_2] = [O_2]^0 \quad \text{at } t = 0 \quad (10)$$

It is worth noting that the oxygen material balance does not account for source terms since the flask was sealed during the disruption reaction. A detailed description of symbol significance is reported in the Section 6.

In order to solve Eqs. (1–3) the concentration of OH· radicals at each integration time should be known. To this aim the system of equations describing the mass balances of all the species appearing in the Fenton's reaction chain of Table 1 should be coupled to the equations above. In this regard, it should be pointed out that, since the rate constants k_3, k_4 and k_5 are order of magnitude smaller than k_2 and k_6 (cf. Table 1), the reactions R3, R4 and R5 can be neglected [19], while the material balance for the OH· radical can be written as follows:

$$\frac{d[OH^{\cdot}]}{dt} = k_1[Fe^{2+}][H_2O_2] - k_2[OH^{\cdot}][H_2O_2] - k_6[OH^{\cdot}][Fe^{2+}] - y_{OH/X}k_d[OH^{\cdot}][X] - k_{L1}[OH^{\cdot}][LH] \quad (11)$$

However, since $OH^{\cdot}$ is a highly reactive free radical with an extremely short life time of nanoseconds, the pseudo steady state assumption can be invoked, i.e. its change rate might be considered to approach zero [19]. Therefore, the time derivative in Eq. (11) can be set equal to zero and the concentration of the $OH^{\cdot}$ radicals can be evaluated for each integration time as follows:

$$[OH^{\cdot}] = \frac{k_1[Fe^{2+}][H_2O_2]}{k_2[H_2O_2] + k_6[Fe^{2+}] + y_{OH/X}k_d[X] + k_{L1}[LH]} \quad (12)$$

As it can be observed from Eq. (12), the concentrations of Fe^{2+} and H_2O_2 should be known at each integration time in order to evaluate the current concentration of $OH^{\cdot}$. To this aim the following material balances for Fe^{2+} and H_2O_2 must be coupled to the equations so far reported:

$$\frac{d[H_2O_2]}{dt} = -k_1[Fe^{2+}][H_2O_2] - k_2[OH^{\cdot}][H_2O_2] - k_3[Fe^{3+}][H_2O_2] \quad (13)$$

$$\frac{d[Fe^{2+}]}{dt} = -k_1[Fe^{2+}][H_2O_2] + k_3[Fe^{3+}][H_2O_2] + k_4[Fe^{3+}][HO_2^{\cdot}] - k_5[Fe^{2+}][HO_2^{\cdot}] - k_6[OH^{\cdot}][Fe^{2+}] \quad (14)$$

According to the literature [19], k_1 and k_3 are much lower than k_2 as well as k_4 and k_5 are much lower than k_6 (cf. Table 1) and thus Eqs. (13) and (14) can be simplified as follows:

$$\frac{d[H_2O_2]}{dt} = -k_2[OH^{\cdot}][H_2O_2] \quad (15)$$

$$\frac{d[Fe^{2+}]}{dt} = -k_6[OH^{\cdot}][Fe^{2+}] \quad (16)$$

along with the following initial conditions:

$$[H_2O_2] = [H_2O_2]^0 \quad \text{at } t = 0 \quad (17)$$

$$[Fe^{2+}] = [Fe^{2+}]^0 \quad \text{at } t = 0 \quad (18)$$

Ultimately the solution of the system of differential Eqs. (1–3) and (11–14), along with Eq. (8), allows one to evaluate the concentration of lipids (LH) in the bulk solution at each operation time during the disruption reaction.

As shown in the materials and methods section, once the desired contact time is elapsed, the reactive phenomena were stopped by diluting ten times the reacting mixture through the addition of ethanol. Subsequently, a solvent extraction step was performed to transfer the lipids from the disrupting solution to an organic solvent phase. Given the high hydrophobic character of lipids, it can be reasonably assumed that all the lipids liberated from microalgal cells during the disruption reaction, and not degraded at the end of the disruption process, could be effectively transferred to the organic solvent phase and then recovered [6]. On the other hand, as shown by Steriti et al. [18], when solvent extraction is performed on undisrupted microalgae, the weight of extracted lipids was equal to about the 7% by unit weight of dried microalgal biomass which was subjected to the extractive procedure. It is important to highlight that such value was confirmed by several repetitions of the extraction procedure on undisrupted biomass and thus represents a quite reliable estimate of the yield of the solvent extraction process on undisrupted biomass. On the basis of such considerations, it can be reasonably inferred that, once the disruption reaction is terminated, the entire amount of lipids LH liberated from the disrupted biomass and not degraded by hydroxyl radicals, can be recovered in the subsequent solvent extraction step. In the case where a residual undisrupted biomass X remained in solution after disruption phase was terminated, the lipids recovered correspondingly are of about 7 %wt/wt of its dry weight. For this reason, the weight percentage of lipids recovered (η_L%) from microalgal biomass, subjected to the disruption procedure under the different operating conditions can be evaluated as follows:

$$\eta_L\% = \frac{[LH]MW_L + 0.07[X]MW_X}{[X]^0 MW_X} \cdot 100 \quad (19)$$

where [LH] and [X] represent the concentration of lipids and undisrupted biomass concentration, respectively, at the end of the disruption reaction, while, the symbols MW_L and MW_X represent the average molecular weight of algal lipids and *C. vulgaris* biomass, respectively. For the sake of brevity the symbol η_L% will be hereafter indicated with the term "extracted lipids". It should be clarified that actually the quantitative description of lipid transfer phenomena from the disrupting solution to the solvent phase would require a dedicated mathematical model which however would be out of the scope of the present work. Moreover Eq. (18) provides an approximate estimate of the extracted lipids which is more reliable the lower is the final residual content of undisrupted biomass, i.e. the lower is the contribution of the second term of Eq. (18) to the value of η_L%. In fact, while the assumption that liberated lipids can be entirely transferred to the solvent phase with an efficiency of almost 100% is a quite realistic hypothesis, on the other hand, the consideration that a fixed percentage weight of lipid (i.e. 7%) could be extracted from undisrupted biomass requires further experimental confirmation. However, from the simulations reported in what follows it was observed that the contribution of the second term of Eq. (18) to the final value of η_L is significant only during the first minute of the reaction while becomes negligible after this period of time and thus Eq. (18) can provide a realistic estimate of the lipids transferred in the solvent phase after disruption.

The system of ordinary differential Eqs. (1–3) and Eqs. (11–14) was numerically integrated as an initial value problem with the Gear method by means of the subroutine DIVPAG of the standard numerical libraries (IMSL). Finally, tuning of model parameters values to fit model results to experimental data was carried out through an optimization procedure which minimize an objective function by means of a Fortran subroutine based on the least-squares method.

4. Results and discussion

The microalgal biomass used to perform the disruption experiments was obtained by cultivation of *C. vulgaris* in a BIOCOIL photo bioreactor fed with pure CO_2 [18]. Once the culture reached steady state, the photobioreactor was operated in fed-batch mode and the wet biomass harvested during each withdrawal cycle was centrifuged and then subjected to the disruption procedure as reported by Steriti et al. [18]. Subsequently, the disrupted biomass was subjected to the lipid extraction procedure in order to verify the effects of the treatment on the amount of lipids which could be extracted from algae. As mentioned above, several disruption experiments were performed where contact time and concentration of disruption reactant were suitably varied in order to identify their corresponding values which allowed maximizing the extracted lipids. The effect of the contact time variation on the amount of extracted lipids when using a disruption solution containing 0.5 mol L^{-1} of H_2O_2 and 0.024 mol L^{-1} of $FeSO_4$ is shown in Fig. 2. It can be observed that when no disruption treatment was performed, i.e. the contact time was zero, extracted lipids were about the 7 %wt/wt by dry weight of biomass. Nevertheless, when the lipid extraction was preceded by a 1 min prolonged disruption treatment the

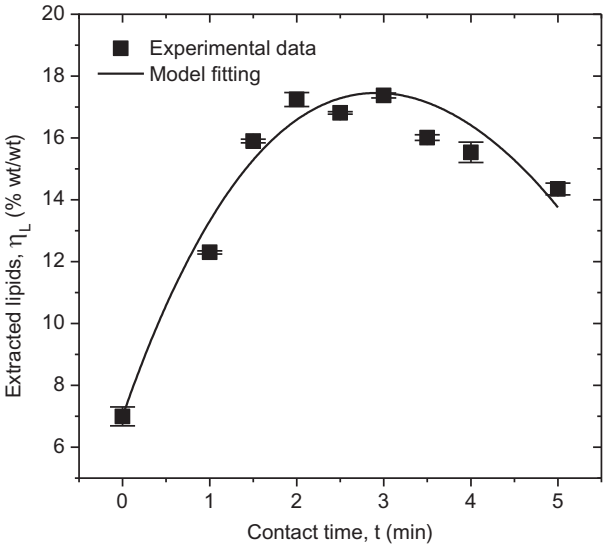

Fig. 2. Comparison of model results and experimental data in terms of lipids extracted from wet biomass subjected to cell disruption treatments performed with an aqueous solution containing 0.5 mol L^{-1} of H_2O_2 and 0.024 mol L^{-1} of $FeSO_4$ by varying the contact time.

extracted lipids increased to about 13.5 %wt/wt. Moreover, when the contact time was further increased, the amount of extracted lipids was correspondingly augmented until a maximum value of 17.4 %wt/wt was attained for a 3 min prolonged disruption treatment. Ultimately, by prolonging the contact time up to 3 min under the operating conditions above specified, the extracted lipids were more than doubled with respect to the case where no disruption was performed. However, when the disruption procedure was protracted for more than 3 min, a decrease of the extracted lipids was observed. According to the reaction mechanisms depicted in the modeling section, such decrease was due to the fact that after 3 min the cell wall degradation was almost completed and residual OH· radicals are able to attack and oxidize lipids transferred in the bulk of the disrupting mixture, thus leading to a decrease of the corresponding amount which was collected in the subsequent extractive step. In Fig. 2, the comparison between experimental data and model results is also shown. While the values of the main rate constants used to perform the simulations are shown in Tables 1 and 2, the remaining model parameters and initial conditions are reported in Table 3. It should be noted from Table 3 that the lipid yield value $y_{L/X}$ ($mol_{lipids}/mol_{biomass}$), which represents the moles of lipids released for each mole of dry biomass being disrupted, has been evaluated by assuming that all lipids contained within the microalgal cell were released in the bulk after cell disruption. Therefore, its value was calculated by assuming an average lipid content q_L of *C. vulgaris* equal to 22 %wt/wt [32–34], through the relationship $y_{L/X} = q_L \cdot (MW_x/MW_L)$ where the symbols MW_L and MW_X represent the average molecular weight of algal lipids and *C. vulgaris* biomass, respectively, whose corresponding values were evaluated on the basis of their average elemental compositions reported in the literature [2,31]. It is important to observe that, in spite of the wide range of values of lipid content available in the literature, the one chosen in this work (i.e. 22%) represents one among the most widespread, and thus representative, as far as the *C. vulgaris* strain is concerned. Moreover, the assumption above is consistent with our preliminary experimental results obtained through the lipid quantification technique based on the use of sulfo phospho vanillin proposed by Cheng et al. [35]. In fact, it is confirmed that the total lipid content of *C. vulgaris* grown in the BIOCOIL under high CO_2 concentration was about 22–23 %wt/wt. Such experimental result somehow confirms the literature values adopted to estimate the whole lipid content of the *C. vulgaris* strain used for the disruption experiments. Furthermore, the value taken from the literature for the rate constant of disruption k_d is referred to the reaction of hydroxyl radicals with generic organic compounds since no value was available for the specific compounds constituting the cell wall of microalgae. Finally it should be noted that the initial concentration of dissolved oxygen $[O_2]^0$ is considered to be equal to the equilibrium value with air at 25 °C. All the parameters values appearing in Table 3 were taken from the literature or experimentally evaluated except for the reaction rate constant of the lipid peroxidation initiation reaction k_{L1} (L mol^{-1} s^{-1}) and the yield $y_{OH/X}$ representing the moles of OH· radicals needed to oxidize one mole of microalgal biomass. The relative error obtained by the fitting procedure was equal to 2.7% when using the values of the tuned parameters shown in Table 3. As it can be observed from Fig. 2, the experimental behavior is well captured by the proposed model, thus confirming that the assumptions made about the chemical physical mechanisms affecting the percent amount of lipid extractable from the unit weight of dry biomass after disruption are consistent with the experimental evidence.

To test the predictive model capability, further experimental data, obtained by performing disruption with growing concentrations of H_2O_2, while keeping fixed the contact time at the value of 3 min, were simulated. Specifically, in such experiments the concentration of $FeSO_4$ was maintained constant at 0.024 mol L^{-1}, while the concentration of H_2O_2 was varied within the range between zero and 5.8 mol L^{-1}. In this regard it should be noted that new experimental data were added with respect to the ones reported by Steriti et al. [18]. From Fig. 3, it can be observed that when H_2O_2 concentration was increased up to 0.5 mol L^{-1}, the extracted lipids were correspondingly increased. However, when the H_2O_2 concentration was further augmented, a reduction of the extracted lipids was observed. As it can be seen from Fig. 3, such experimental behavior is quite well predicted by the proposed model. It should be remarked that model parameters used in this simulation run were the same reported in Table 3 and thus no parameter was adjusted. Ultimately, the proposed model allows to properly simulate the weight percentage of lipids which can be recovered from the microalgal biomass subjected to the disruption procedure with different H_2O_2 concentrations and contact times. Therefore, the model represents a first step towards the development of a design and control tool which allows optimizing the investigated disruption technique. Such kind of tools are critical in view of implementing the technique at the industrial scale since, as it can be observed from the figures, the extraction yields are quite sensitive to the operating conditions adopted during disruption and hence they should be properly set in order to achieve the

Table 3
Model parameters and initial conditions.

Symbol	Value	Units	References
$[Fe_{2+}]^0$	0.024	mol L^{-1}	[18]
$[H_2O_2]^0$	0.00–5.80	mol L^{-1}	[18]
k_d	5.00×10^8	L mol^{-1} s^{-1}	[20,30]
k_{L1}	5.44×10^7	L mol^{-1} s^{-1}	Tuned parameter
$[L\cdot]^0$	0.00	mol L^{-1}	[18]
$[LH]^0$	0.00	mol L^{-1}	[18]
$[LO_2]^0$	0.00	mol L^{-1}	[18]
$[LO_2H]^0$	0.00	mol L^{-1}	[18]
MW_L	6.34×10^2	g mol^{-1}	[31]
MW_X	20.14×10^3	g mol^{-1}	[2]
$[O_2]^0$	2.58×10^3	mol L^{-1}	Evaluated from [18]
$[X]^0$	3.97×10^{-3}	mol L^{-1}	[18]
$y_{L/X}$	6.99	mol mol^{-1}	Evaluated from [32–34]
$y_{OH/X}$	2.27×10^4	mol mol^{-1}	Tuned parameter

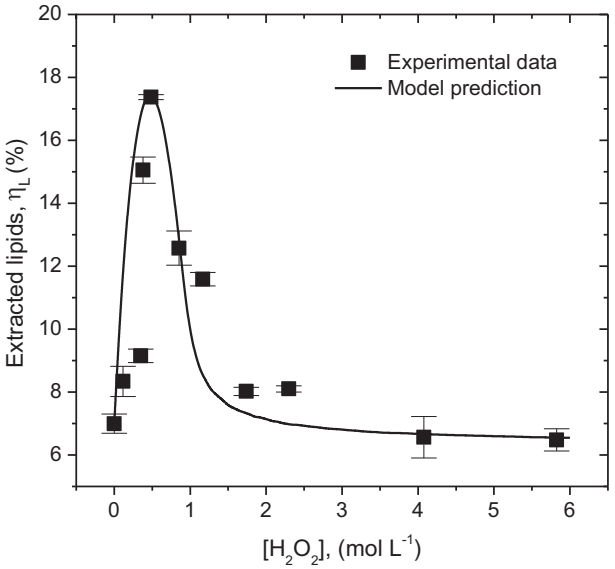

Fig. 3. Comparison of model results with experimental data in terms of lipids extracted from wet biomass subjected to cell disruption treatments performed with aqueous solutions containing concentrations of $FeSO_4$ kept fixed to 0.024 mol L^{-1} and H_2O_2 concentrations up to 6 mol L^{-1}, while maintaining the contact time at 3 min.

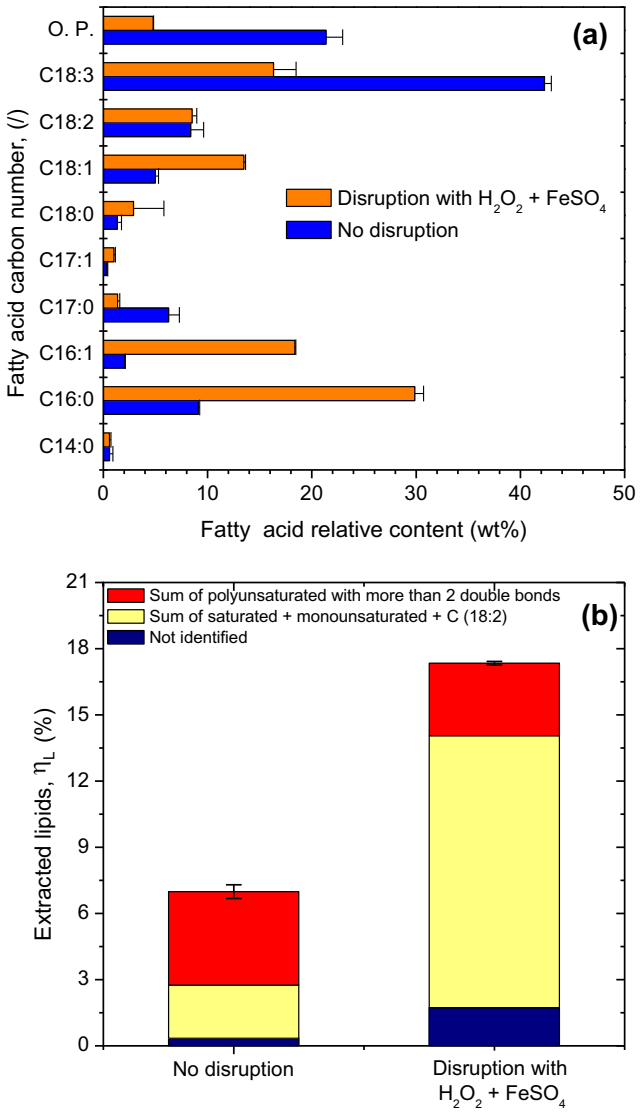

Fig. 4. Comparison of fatty acid methyl esters profile of lipids extracted from undisrupted microalgae and the corresponding ones extracted after performing cell disruption under the optimal operating conditions. The acronym O.P. indicates the sum of other polyunsaturated fatty acids having more than 2 double bonds.

desired effect of the treatment, while avoiding the undesired oxidation of extracted lipids. In this regard it should be clarified that, while the lipid oxidation phenomena can be minimized when operating under optimal conditions, in any case, a certain amount of lipids released from the algal cell after disruption is subjected to the attack of hydroxyl radicals and thus is irreversibly degraded to useless products. By considering for example the investigated case study, while starting from a lipid content equal to 22 %wt/wt, the amount of lipid extracted under optimal conditions is at most equal to 17.5 %wt/wt (cf. Figs. 2 and 3). It follows that, at least an aliquot of 4.5 %wt/wt of lipids originally contained within the microalgae cell are degraded due to the oxidative phenomena. While from one hand such phenomenon might be seen as a drawback of the proposed technique since it results in the incomplete availability of the whole lipid content of microalgae, from the other one it is probably the main responsible of the significant improvement of the quality of the biodiesel obtained from microalgae lipids observed by Steriti et al. [18] as a result of the disruption treatment. In fact, as it can be observed from Fig. 4a, the analysis of the fatty acid methyl esters (FAMEs) obtained through trans-esterification of the lipids extracted from microalgae, highlighted that the disruption treatment provoked a dramatic reduction of the relative content of undesired polyunsaturated fatty acids (i.e. with more than 2 double bonds) and, in particular, a significant decrease of linolenic fatty acid (C18:3) with respect to the case where disruption was not performed before lipid extraction. Specifically, the relative content of linolenic acid was reduced from the value of 42 %wt, in absence of disruption, to the value of 16.3 %wt/wt when disruption was previously carried out. Correspondingly, the sum of the other polyunsaturated fatty acids (O.P. in Fig. 4a) was reduced from the value of 21 %wt/wt to the one of 4.75 %wt/wt when the disruption treatment was performed. The above characteristics make the biodiesel obtainable from disrupted biomass more stable than the corresponding one from undisrupted biomass. In fact an high content of poly-unsaturated FAMEs results in a higher tendency of biodiesel to oxidize and degrade during storage under the action of air, light, heat, trace metals, etc., during storage. Ultimately, the latter results highlighted that a significant improvement of the final quality of lipid extracted was provoked by the disruption treatment, along with the already shown increase of the amount of extracted lipids (cf. Fig. 4b). In fact, the saturated acids as well as the monounsaturated ones and the linoleic acid (C18:2), whose relative content is increased as a result of the disruption treatment (cf. Fig. 4b), represent the most desirable class of fatty acids for the production of biodiesel. Most probably, such improvement was due just to the fact that an aliquot of algal lipids released from the cell during disruption are oxidized by the $OH^{\cdot}$ radicals. In fact, as already mentioned in the description of the mathematical model, the peroxidation phenomena involve preferably the polyunsaturated fatty acids, including the linolenic one, since they display multiple double bonds whose weakly bound hydrogen atoms can be easily abstracted by the $OH^{\cdot}$ radicals produced by the Fenton reaction, as shown in Fig. 1 [27,28]. Therefore, while from one hand the peroxidation reactions can actually provoke a degradation of lipids liberated from the cell, from the other side they involve mainly the most undesired category of lipids for producing biodiesel, i.e. the polyunsaturated ones, thus providing a better quality of the resulting oil in view of its exploitation for

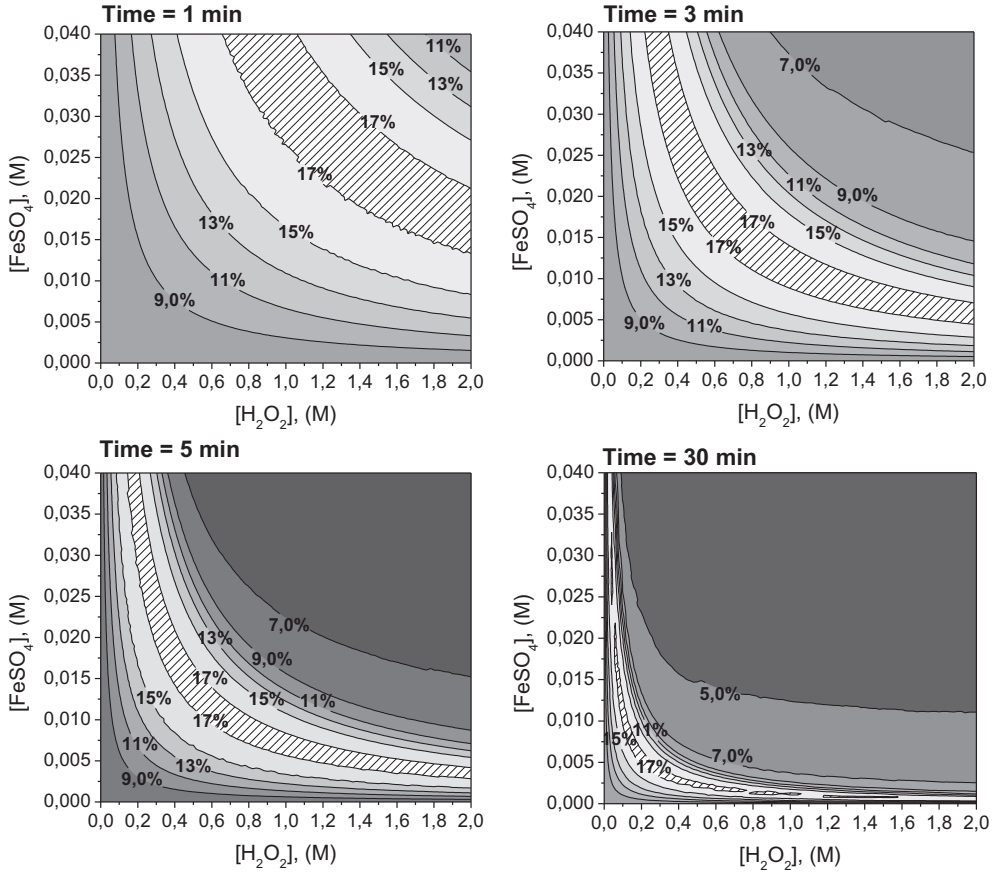

Fig. 5. Optimization maps, obtained through the mathematical model, showing the simulated values of lipids extracted (η_L%) from microalgal biomass when performing disruption with specific couples of values of and H_2O_2 and $FeSO_4$ concentrations and different contact times.

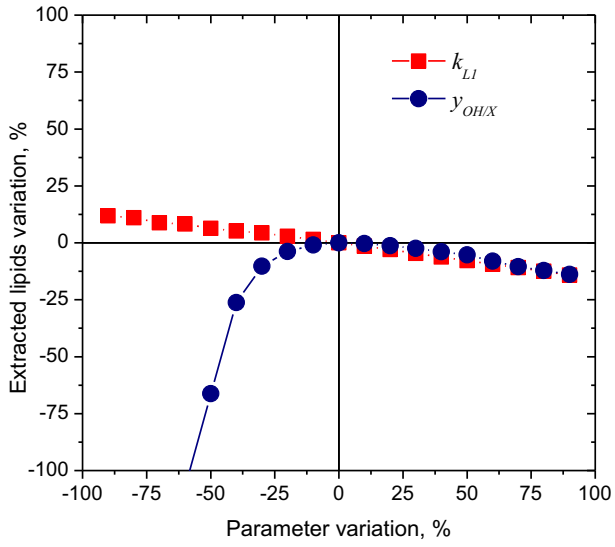

Fig. 6. Sensitivity analysis in terms of percentage variation of extracted lipids with respect to the value obtained under optimal operating conditions, i.e. η_L = 17.4%, when the considered parameters values are modified up to ±100% with respect to their corresponding values reported in Table 3.

producing biofuels. Moreover, for the sake of clarity, it should be specified here that, not all the lipids were converted into FAMEs by the transesterification reaction. In particular, only an amount equal to 95 and 90 %wt/wt for the case of lipids extracted from undisrupted and disrupted biomass, respectively (cf. Fig. 4b) has been identified. While this fact is quite usual when performing transesterification of algal oils, it should not be neglected when designing industrial scale reactors in order to avoid over-estimation of the biodiesel production capacity.

Nevertheless, although the proposed technique involves this positive side effect, it is apparent that peroxidation phenomena should be limited in order to avoid a too pronounced degradation of the useful lipids released from the cell. In fact, it can observed from Figs. 2 and 3 that, whether the disruption treatment is prolonged in time or the concentration of disruption reactants is too high, the extracted lipids might be dramatically decreased up to values lower than the corresponding ones achievable without disruption treatment. Therefore, both reactants concentration and contact time should be finely tuned in order to achieve the optimal compromise between amount of extracted lipids and their final composition in terms of FAMEs. To this aim the proposed model allows to assess "a priori" the effect of varying these operating conditions on the final extracted lipids. An example of how the model might be used to identify the operating conditions which allow achieving the desired outcomes is shown in Fig. 5 wherein modeling results are reported in the form of contour maps showing the values of extracted lipids obtained by performing several simulations with different values of initial concentrations of $FeSO_4$ and H_2O_2 as well as different values of the contact time. It can be observed that the highest values of extracted lipids, i.e. those corresponding to the patterned regions in the graphs, might be obtained using different combinations of concentration values of Fe_2SO_4, H_2O_2 and contact time. In particular, when short contact times are considered, high values of the disruption reactants

concentration should be used to get the optimum value of extracted lipids (i.e. >17.4 %wt/wt). On the other hand, if the contact time is gradually prolonged, the same goal might be achieved by using lower concentration of reactants. Therefore, whether the disruption technique would be implemented at the industrial scale, the proposed model might permit the choice of reducing the operating costs associated to the purchase of reactants by increasing the contact times or, on the contrary, reducing the volume of the disruption reactor by decreasing the contact time. Moreover, since the contour lines of Fig. 5 show an hyperbolic profile, depending on the costs and the availability of reactants, one might eventually choose to select high concentration of $FeSO_4$ and low concentration of H_2O_2 or vice versa while keeping fixed the optimal extraction yield.

While the considerations above aim to highlight the capability of the developed model to aid the design of the disruption process, it should be clarified that, in order to develop a robust mathematical tool to be used for industrial applications, the present model should be further validated through comparison with new experimental data obtained under a wide range of operating conditions. However, at least under the investigated operating conditions and using the set of model parameters of Tables 1–3, the proposed model is proven to be quite reliable and permitted to confirm the assumptions made by [18] about the chemical physical mechanisms underlying the relevant experimental results obtained through the novel disruption technique proposed.

Finally, a sensitivity analysis was performed to assess the effect of tunable parameters ($y_{OH/X}$ and k_{L1}) variation on the final extracted lipids. The results of such analysis are reported in Fig. 6 in terms of percentage variation of extracted lipids with respect to the value obtained under optimal operating conditions, i.e. η_L = 17.4%, when the considered parameters values are modified up to ±100% with respect to their corresponding values reported in Table 3. It can be observed that the parameter k_{L1}, indicating the rate constant of the initiation step of the lipid oxidation reactive chain, does not seem to significantly affect the considered model output thus indicating that the obtained value is quite reliable. In fact, a maximum output variation of ±10% is observed when the k_{L1} value is correspondingly varied by ±100% with respect to its corresponding value reported in Table 3. On the contrary, the model shows a high sensitivity to negative variations of the parameter $y_{OH/X}$. In fact the extracted lipids varied more than −100% when the parameter value is reduced of about −60% with respect to its corresponding value of Table 3. This result is due to the fact that a low value of $y_{OH/X}$ implies that few moles of OH· radicals are sufficient to complete microalgal cell disruption. As a result, the cell disruption process is completed in short period of time and the higher residual concentration of OH· in solution can attack more effectively the released lipids which in turn are almost completely degraded to useless products. These results call for the need for further validation of the adopted value for the parameter $y_{OH/X}$. In particular the obtained value (cf. Table 3) should be confirmed through the fitting of further experimental trials as well as of new experimental outputs such as the evolution of Fe^{2+} and intact cells (X) concentration during disruption. Work is on the way along these lines.

5. Concluding remarks

A mathematical model for the simulation of the effect of a cell disruption treatment based on Fenton reaction on the amount of lipids extractable from *C. vulgaris* is proposed. By comparing model results with literature experimental data a good matching is obtained. Moreover, a possible explanation of how the disruption treatment may have influenced the improvement of FAMES composition observed in the literature is proposed. The model might represent the first step towards the development of a software tool useful to optimize the implementation of the disruption technique at the industrial scale.

6. Notations

Symbol	Significance
[DX]	Concentration of disrupted algae (mol L^{-1})
k_i	Constant rates for the Fenton reactions, i = 1,..6 (L mol^{-1} s^{-1})
k_d	Constant rate of the disruption reaction (L mol^{-1} s^{-1})
k_{Li}	Constant rates of the lipid peroxidation reactions, i = 1,..4 (L mol^{-1} s^{-1})
[LH]	Concentration of lipids (mol L^{-1})
[L·]	Concentration of lipid radicals (mol L^{-1})
[LO_2·]	Concentration of lipid peroxyl radicals (mol L^{-1})
[LO_2H]	Concentration of lipid peroxides (mol L^{-1})
MW_L	Molar mass of algal lipids (g mol^{-1})
MW_X	Molar mass of *C. vulgaris* (g mol^{-1})
t	Time (s)
$y_{OH/X}$	Moles of OH· needed to disrupt one mol of algal biomass (mol mol^{-1})
$y_{L/X}$	Moles of lipids released for each mol of algae disrupted (mol mol^{-1})
[X]	Microalgae concentration (mol L^{-1})
Greek letters	
η_L	Weight of lipids extracted for unit dry weight of microalgal biomass subjected to disruption (% wt/wt)
Subscripts	
d	Disruption (–)
L	Lipids (–)
Superscripts	
0	Initial condition (–)

Acknowledgement

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References

[1] A. Concas, M. Pisu, G. Cao, Novel simulation model of the solar collector of BIOCOIL photobioreactors for CO_2 sequestration with microalgae, Chem. Eng. J. 157 (2010) 297–303.
[2] A. Concas, G.A. Lutzu, M. Pisu, G. Cao, Experimental analysis and novel modeling of semi-batch photobioreactors operated with *Chlorella vulgaris* and fed with 100% (v/v) CO_2, Chem. Eng. J. 213 (2012) 203–213.
[3] E. Jacob-Lopes, T.T. Franco, From oil refinery to microalgal biorefinery, J. CO_2 Utilization 2 (2013) 1–7.
[4] A. Concas, A. Steriti, M. Pisu, G. Cao, Comprehensive modeling and investigation of the effect of iron on the growth rate and lipid accumulation of *Chlorella vulgaris* cultured in batch photobioreactors, Bioresour. Technol. 153 (2014) 340–350.
[5] R. Halim, T.W. Rupasinghe, D.L. Tull, P.A. Webley, Modelling the kinetics of lipid extraction from wet microalgal concentrate: a novel perspective on a classical process, Chem. Eng. J. 242 (2014) 234–253.
[6] B.H. Yap, S.A. Crawford, G.J. Dumsday, P.J. Scales, G.J. Martin, A mechanistic study of algal cell disruption and its effect on lipid recovery by solvent extraction, Algal Res. 5 (2014) 112–120.

[7] J. Kim, G. Yoo, H. Lee, J. Lim, K. Kim, C.W. Kim, M.S. Park, J.W. Yang, Methods of downstream processing for the production of biodiesel from microalgae, Biotechnol. Adv. 31 (2013). 962-876.
[8] R. Halim, R. Harun, M.K. Danquah, P.A. Webley, Microalgal cell disruption for biofuel development, Appl. Energy 91 (2012) 116–121.
[9] A.K. Lee, D.M. Lewis, P.J. Ashman, Force and energy requirement for microalgal cell disruption: an atomic force microscope evaluation, Bioresour. Technol. 128 (2013) 199–206.
[10] J.R. McMillan, I.A. Watson, M. Ali, W. Jaafar, Evaluation and comparison of algal cell disruption methods: microwave, waterbath, blender, ultrasonic and laser treatment, Appl. Energy 103 (2013) 128–134.
[11] A.P. Florentino de Souza, M.C. Costa, A. Colzi Lopes, E. Fares Abdala Net, R. Carrhá Leitão, C.R. Mota, A. Bezerra dos Santos, Comparison of pretreatment methods for total lipids extraction from mixed microalgae, Renew. Energy 63 (2014) 762–766.
[12] N. Grimi, A. Dubois, L. Marchal, S. Jubeau, N.I. Lebovka, E. Vorobiev, Selective extraction from microalgae *Nannochloropsis* sp. using different methods of cell disruption, Bioresour. Technol. 153 (2014) 254–259.
[13] U.D. Keris-Sen, U. Sen, G. Soydemir, M.D. Gurol, An investigation of ultrasound effect on microalgal cell integrity and lipid extraction efficiency, Bioresour. Technol. 152 (2014) 407–413.
[14] M. Wang, W. Yuan, X. Jiang, Y. Jing, Z. Wang, Disruption of microalgal cells using high-frequency focused ultrasound, Bioresour. Technol. 153 (2014) 315–321.
[15] G. Young, F. Nippen, S. Titterbrandt, M.J. Cooney, Direct transesterification of biomass using an ionic liquid co-solvent system, Biofuels 2 (2011) 261–266.
[16] X. Bai, F. Ghasemi Naghdi, L. Ye, P. Lant, S. Pratt, Enhanced lipid extraction from algae using free nitrous acid pretreatment, Bioresour. technol. 159 (2014) 36–40.
[17] A. Sathish, R.C. Sims, Biodiesel from mixed culture algae via a wet lipid extraction procedure, Bioresour. Technol. 118 (2012) 643–647.
[18] A. Steriti, R. Rossi, A. Concas, G. Cao, A novel cell disruption technique to enhance lipid extraction from microalgae, Bioresour. technol. 164 (2014) 70–77.
[19] Y. Wu, S. Zhou, F. Qin, K. Zheng, X. Ye, Modeling the oxidation kinetics of Fenton's process on the degradation of humic acid, J. Hazard. Mater. 179 (2010) 533–539.
[20] N. Kang, D.S. Lee, J. Yoon, Kinetic modeling of Fenton oxidation of phenol and monochlorophenols, Chemosphere 47 (2002) 915–924.
[21] J.J. Pignatello, E. Oliveros, A. MacKay, Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related chemistry, Crit. Rev. Env. Sci. Technol. 36 (2006) 1–84.
[22] S. Wang, A comparative study of Fenton and Fenton-like reaction kinetics in decolourisation of wastewater, Dyes Pigm. 76 (2008) 714–720.
[23] J.M. Rosas, F. Vicente, E.G. Saguillo, A. Santos, A. Romero, Remediation of soil polluted with herbicides by Fenton-like reaction: kinetic model of diuron degradation, Appl. Catal. B 144 (2014) 252–260.
[24] A.R. Fajardo, L.E. Cerdan, A.R. Medina, F.G.A. Fernández, P.A.G. Moreno, E.M. Grima, Lipid extraction from the microalga *Phaeodactylum tricornutum*, Eur. J. Lipid Technol. 109 (2007) 120–126.
[25] EEC 2568/91, Characteristics of olive and olive pomance oils and their analytical methods, Off. J. Eur. Communities L248 (1991) 1–82.
[26] M.J. Flores, R.J. Brandi, A.E. Cassano, M.D. Labas, Chemical disinfection with H_2O_2 – the proposal of a reaction kinetic model, Chem. Eng. J. 198 (2012) 388–396.
[27] O.K. Dalrymple, E. Stefanakos, M.A. Trotz, D.Y. Goswami, A review of the mechanisms and modeling of photocatalytic disinfection, Appl. Catal. 98 (2010) 27–38.
[28] A.B. Doktorov, N.N. Lukzen, J.B. Pedersen, Analysis of lipid peroxidation kinetics. 1. Role of recombination of alkyl and peroxyl radicals, J. Phys. Chem. B 112 (2008) 11854–11861.
[29] C. Xue, C.S. Chou, C.Y. Kao, C.K. Sen, A. Friedman, Propagation of cutaneous thermal injury: a mathematical model, Wound Repair Regen. 20 (2012) 114–122.
[30] F.J. Rivas, F.J. Beltrán, O. Gimeno, J. Frades, Treatment of olive oil mill wastewater by Fenton's reagent, J. Agric. Food Chem. 49 (2001) 1873–1880.
[31] L. Lardon, A. Hélias, B. Sialve, J.P. Steyer, O. Bernard, Life-cycle assessment of biodiesel production from microalgae, Environ. Sci. Technol. 43 (2009) 6475–6481.
[32] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2006) 87–96.
[33] H. Zheng, J. Yin, Z. Gao, H. Huang, X. Ji, C. Dou, Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves, Appl. Biochem. Biotechnol. 164 (2011) 1215–1224.
[34] G. Mujtaba, W. Choi, C.G. Lee, K. Lee, Lipid production by *Chlorella vulgaris* after a shift from nutrient-rich to nitrogen starvation conditions, Bioresour. Technol. 123 (2012) 279–283.
[35] Y.S. Cheng, Y. Zheng, J.S. VanderGheynst, Rapid quantitative analysis of lipids using a colorimetric method in a microplate format, Lipids 46 (2011) 95–103.