Effectiveness of *Parachlorella kessleri* cell disruption evaluated with the use of laser light scattering methods

Agata Piasecka¹ · Jolanta Cieśła¹ · Magdalena Koczańska¹ · Izabela Krzemińska¹

Received: 28 May 2018 / Revised and accepted: 11 July 2018 / Published online: 25 July 2018
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Abstract

The main objective of this study is to demonstrate the possibilities of using laser light scattering methods, dynamic light scattering and laser Doppler electrophoresis, as suitable methods in investigations of algal production biosystems and biotechnology. This paper highlights the innovative use of the dynamic light scattering (DLS) methods for monitoring the destruction of *Parachlorella kessleri* cells. Additionally, these results indicate electrophoretic mobility as a new parameter to investigate the effectiveness of cell disruption prior to extraction conducted to optimise the biotechnological processes of recovery of microalgal intracellular metabolites. The efficacy of *P. kessleri* cell disintegration by ultrasound was determined by measurements of the number of cells with the algal cell reduction (CR_{ns}), relative mean hydrodynamic diameter (Rd_i) and electrophoretic mobility after applying different lengths of ultrasound exposure to a cell suspension. It was found that stationary-phase cells were the most resistant to the ultrasound treatment, especially at low values of the optical density. Both the relative hydrodynamic diameter and the electrophoretic mobility of cells were correlated statistically significantly with the time of sonication (t) and the algal cell reduction. The relationships allowed estimation of the sonication time needed for total cell disruption.

Keywords *Parachlorella kessleri* · Laser light scattering methods · Cell disruption · Sonication

Introduction

Laser light scattering methods can be used for particle characterisation. Light scattering has a broad spectrum of applications, e.g. in cosmology, meteorology, oceanography and nanotechnology, as evidenced in the large number of scientific papers. Laser light scattering methods are applied in biomolecules studies, especially in characterisation of physical and physicochemical properties of bacteria and viruses (Xu 2015; Cieśła et al. 2018).

Algal bioproduction processes are expanded in response to the rapidly increasing demand for medicines, dietary supplements and biofuels. Thus, the research efforts have focused especially on improving the biomass production and extraction of metabolites (Piasecka et al. 2014; García-Galán et al. 2018). Microalgae synthesise many valuable metabolites, e.g. pigments, carbohydrates, amino acids and fatty acids, which are attractive for commercial exploitation (Krzemińska et al.

2015a; Grudziński et al. 2016). Since these compounds are usually intracellularly located, cell disintegration prior to extraction thereof must be carried out (Postma et al. 2017). An effective process of microalgal cell disruption is one of the major challenges for the commercialisation of bioproducts from microalgae.

One of the more recent achievements in algal extraction technology has been the use of physical cell disruption method—ultrasonication; in many studies published today, it is one of the most effective cell disruption methods improving the extraction process (Prabakaran and Ravindran 2011; Zheng et al. 2011; Piasecka et al. 2014).

The destruction of cell walls and cell membranes contributes to the inactivation of the microorganisms and is the basis for the release of cellular contents to the environment. The method employed for cell disruption determines the quality and yields of the intracellular metabolites. The efficiencies of the cell wall disruption methods depend on cell wall strength (Joyce et al. 2014). The composition and structure of the microalgal cell wall varies among strains. The structure of the cell wall varies in the organisation with thickness depending on cell growth and cell division (Yamamoto et al. 2005). The construction of microalgal cell wall makes microalgae less permeable and resistant to extraction.

✉ Izabela Krzemińska
i.krzeminska@ipan.lublin.pl

¹ Institute of Agrophysics, Polish Academy of Sciences,
Doświadczalna 4, 20-290 Lublin, Poland

Parachlorella kessleri is a unicellular freshwater alga (Chlorophyta), which is extensively used for physiological and biochemical studies due to its ability to accumulate starch, lipid and protein and its biotechnological importance (Magierek and Krzemińska, 2017; Piasecka et al. 2017; Lv et al. 2018; Taleb et al. 2018). Depending on the sugar composition of the rigid cell wall, Chlorophyta species are divided into two groups: glucosamine type and glucose-mannose type. *Parachlorella kessleri* has glucosamine as the main sugar component of the rigid cell wall (Takeda 1991). Investigations conducted by Yamamoto et al. (2005) have shown that *P. kessleri* has an electron-transparent thick wall.

Many investigators have used different methods to measure cell disintegration after ultrasonication. These include the diffusion behaviour of proteins and pigments of *Chlorella vulgaris* in the aqueous phase (Safi et al. 2015), reductions in intact cell counts and reductions in average colony diameters (Halim et al. 2012), counting only intact cells with a haemocytometer (Greenly and Tester 2015), changes in the algal cell concentration, cell size, chlorophyll *a* fluorescence and Nile Red-stained lipid fluorescence (Wang et al. 2014) and cell disruption efficiency and lipid release (Natarajan et al. 2014).

Development of the method for measurement of algal cell lysis plays a key role in algal cell disruption and metabolites acquiring. Optical microscopy techniques are commonly used to evaluate the degree of cell disintegration. However, there have been no reports so far on the use of laser light scattering measurements in assessing the degree of algal cell degradation in a solution in laboratory practice.

The main objective of this study was to apply laser light scattering techniques in investigations of cell wall disruption to determine the efficacy of ultrasonic cell disruption. Additionally, the aim of this work was to assess the impact of sonication treatment on disruption of *P. kessleri* cells depending on the different physiological states of cells at different optical densities.

Material and methods

Microalgal strain and culture conditions

The axenic culture of *Parachlorella kessleri* (No. 250) was obtained from the Culture Collection of Autotrophic Organisms (CCALA) at Charles University in Prague. Cultivation of *P. kessleri* was carried out in a Kessler medium. The Kessler medium is composed (per litre distilled water) of 0.81 g KNO₃, 0.47 g NaCl, 0.47 g NaH₂PO₄·2H₂O, 0.36 g Na₂HPO₄·12H₂O, 0.25 g MgSO₄·7H₂O, 0.014 g CaCl₂·2H₂O, 0.006 g FeSO₄·7H₂O, 0.0005 g MnCl₂·4H₂O, 0.0005 g H₃BO₃, 0.0002 g ZnSO₄·7H₂O, 0.00002 g (NH₄)₆Mo₇O₂₄·4H₂O, and 0.008 g EDTA (Piasecka et al. 2017).

Cultivations were conducted in 500 mL Erlenmeyer flasks at 26 °C under continuous illumination (80 μmol photons m⁻² s⁻¹) with shaking (100 rpm) without aeration. The experiment was carried out in triplicate (*n* = 3).

Growth measurements and biomass determination

Growth was daily monitored (for 15 days) by measuring optical density at 650 nm (Krzemińska et al. 2015b). The biomass concentration was estimated by dry weight determination. Dilution series of the cell suspension were made and optical density (OD₆₅₀) was measured. The cell suspension was filtered through a pre-weighted Whatman GF/C glass-fibre filter and dried at 90 °C for 24 h to constant weight. Based on the calibration curve, which showed the relationship between the optical density at 650 nm and the dry cell weight, the optical values were converted to dry weight by the following Eq. (1):

$$\text{Dry cell weight (DCW)} = 372.21 \times \text{OD}_{650} - 9.1591, \quad (1)$$

$$R^2 = 0.9968.$$

Preparation for laser light scattering measurements

During the cultivation, 100 mL of culture were collected after 24, 96, 240, and 360 h. Each sample represented a different physiological state of *P. kessleri* cells (growth phases as follows, 24 h—Lag phase, 96 h—Log phase, 240 h—Decline phase, 360 h—Stationary phase). Sampling points were marked on the growth curve (Fig. 1). Cells were centrifuged (Rotanta 460 RS, centrifuge) and washed twice with filtered (Whatman Anodisc 0.02 μm) and distilled water (*η* = 1.0031 cP, *n* = 1.330). The microalgal cells were diluted using the abovementioned water to different cell densities (0.05, 0.075, and 0.15 value of optical density at 650 nm—a measuring range limited by the Zetasizer Nano ZS apparatus). The pH value of distilled water was 6.54 ± 0.02 at the

Fig. 1 Growth curve of *P. kessleri* (the results are presented as the means of nine measurements from *n* = 3, error bars represent standard deviation, sampling points are marked with empty dots)

measurement temperature. The microalgal cell suspension (10 mL) in 30-mL glass vials was treated by sonic waves for 10, 20, 30, 40, 50, 60, 90, 180, and 360 s (power 500 W, frequency 20 kHz and amplitude 50%) to disrupt the cells. Sonication was performed using a Sonics vibra cell 500 sonicator. Additionally, a non-sonicated sample was used as a control for each study to enable comparisons.

Cell numbers

After each sonication treatment, the cells were counted under an OLIMPUS microscope with a Bürker haemocytometer (BLAUBRAND counting chambers) CE-marked according to IVD-Directive 98/79 EC.

Algal cell reduction

The algal cell reduction exhibited a decrease in cell viability as a percentage. The algal cell reduction was calculated using the following Eq. (2):

$$CR_{ns} (\%) = 100 - ((CN_{ns} \times 100) / CN_{0s}) \quad (2)$$

where:

CR_{ns} algal cell reduction after n seconds of sonication
 CN_{ns} number of cells counted after n seconds of sonication
 CN_{0s} number of cells counted in the control sample without sonication

Determination of the particle size in algal dispersion

The dynamic light scattering (DLS) method was used for evaluation of the changes in the particle size after the application of ultrasounds to the algal dispersion. Measurements were performed using the Zetasizer Nano ZS apparatus (Malvern Ltd., UK) equipped with a He-Ne (633 nm) source of laser light. The non-invasive back scattering (NIBS) technique and phase analysis light scattering (PALS) were employed for determination of the diameter of the particles (Zetasizer Nano Series User Manual, 2004). Time-dependent fluctuations of the signal were the basis for the autocorrelation function $G_2(\tau)$, which decreases in the time range τ (Kaszuba et al. 2008; ISO 22412:2017):

$$G_2(\tau) = A[1 + B \exp(-2\Gamma\tau)], \quad (3)$$

where: A —constant, which is time-independent and proportional to the square of the mean intensity of scattered light, B —the apparatus coefficient ($B \leq 1$) and Γ —speed of autocorrelation function decay, which for isotropic spherical particles are described by eq. (4):

$$\Gamma = Dq^2, \quad (4)$$

where:

$$q = \frac{4\pi n}{\lambda_0} \sin(\theta/2) \quad (5)$$

is a modulus of the scattering vector, n —refractive index of the dispersant, λ_0 —length of the laser beam wave in vacuum and θ —angle of laser light scattering. The relationship between the diffusion coefficient D and the particle hydrodynamic diameter d is defined by the Stokes-Einstein Eq. (6):

$$D = \frac{kT}{3\pi\eta d}, \quad (6)$$

where.

k is Boltzmann constant
 T absolute temperature
 η solvent viscosity

Measurements were done using the “size & zeta potential” folded capillary cells in 12 sub-runs at the temperature of 20 °C. The general-purpose model of the analysis was applied. Two repetitions of the measurement were performed for each of the three biological replications of samples with appropriate optical density (OD_{650} 0.050; 0.075 and 0.150).

Before the start of the DLS experiment, the samples were observed under an optical microscope to be sure that the size of algal cells was in the range of the apparatus scale (i.e. 0.6–6.0 μm). An example of the photos obtained is presented in Fig. 2. Generally, the cells were circular but their size was at the upper limit of the measuring range of the Zetasizer apparatus.

The DLS method is often used for characterisation of colloidal systems (Sood 1987; Metzeler et al. 2011; Nawrocka and Cieřla 2013; Sharifi 2014). However, for soft materials,

Fig. 2 Photograph of *P. kessleri* cells in the water suspension (OD_{650} = 0.150) before the sonication treatment in light microscope

there may be problems with the sharpness of the particle/dispersant optical interface, which affect the results (Cieřla et al. 2013). Some researchers pointed that, in the case of algal cells, the light is scattered not only by the outside surface but also by the cell interior (Witkowski et al. 1993; Quirantes and Bernard 2004). For concentrated systems, it is possible that the effect of restricted diffusion occurs. This may be the cause of the obtaining of overestimated results (Pecora 1985; ISO 22412:2017). To avoid these interferences, the influence of the sonication time on the algal dispersions was analysed using the relative mean hydrodynamic diameter (Rd_t):

$$Rd_t = \frac{d_t}{d_0} \quad (7)$$

where d_t and d_0 were the mean hydrodynamic diameters at time t and $t = 0$ s, respectively.

Electrophoretic mobility of algal cells

Electrophoretic mobility characterises the movement of particles under the influence of an electric field applied:

$$U_e = \frac{v}{E} = \left(\frac{\varepsilon \zeta}{\eta} \right) f(\kappa a) \quad (8)$$

where U_e —electrophoretic mobility, v —velocity of dispersed particles, E —electric field strength, ε —permittivity of the dispersing medium, ζ —zeta potential, η —viscosity of the dispersing medium and $f(\kappa a)$ —Henry's function (Hunter 1988; Zetasizer Nano Series User Manual, 2004; Delgado et al. 2007).

The values of electrophoretic mobility are often used for determination of electrokinetic potential to evaluate the stability of the colloidal systems (Hunter 1988; Delgado et al. 2007; Wu et al. 2012; Ndikubwimana et al. 2015). In these investigations, the electrophoretic mobility was measured using the Zetasizer Nano ZS apparatus (Malvern Ltd., UK) and the laser Doppler electrophoresis (LDE) method (Mayering 1994; Zetasizer Nano Series User Manual, 2004). Measurements were done using the same cells and conditions as in the DLS method.

Statistical analysis

To determine the influence of the sonication time, physiological stage and OD_{650} on the cell number, particle size (mean hydrodynamic diameter— d), relative mean hydrodynamic diameter (Rd_t) and electrophoretic mobility, the multi-factor ANOVA (at $p < 0.05$), one-way ANOVA and the post hoc Tukey's HSD test were used (Statistica v. 10.0, StatSoft Poland). The Statistica software was applied to find the correlations (at $p < 0.05$) between Rd_t and the sonication time (t), Rd_t and the algal cell reduction (CR_{ns}), electrophoretic

mobility and sonication time (t), as well as electrophoretic mobility and the algal cell reduction (CR_{ns}).

The particle size, Rd_t , and electrophoretic mobility data presented in the figures and tables are arithmetic means of six replicates with the indication of standard deviation. In the case of the dry cell weight and cell number, the mean results and standard deviations were obtained from nine and six replicates, respectively.

Results

The present results show changes in the number of *P. kessleri* cells during sonication at three different optical densities (OD_{650}) and at four physiological stages (Fig. 3). The effectiveness of ultrasound presented as the number of cells was significantly dependent on the time of exposition to the sonication, OD_{650} value and growth phase ($p < 0.05$). In this study, the increase in the sonication exposure time ranging from 10 to 360 s caused a decrease in the number of viable cells in the solution in comparison with the control. The application of ultrasonic waves for 360 s did not result in total cell disintegration: the solution still contained viable cells (Table 1).

The effect of the ultrasound treatment on cell disintegration expressed as CR_{ns} in the lag, log, decline and stationary phases after 360 s of exposure was 72.84, 46.96, 54.21 and 9.27%, respectively, at 0.05 OD; 58.17, 49.60, 49.78 and 28.99% at 0.075 OD and 52.85, 61.14, 44.57 and 38.61% at 0.15 OD. An important effect was noted wherein the increasing OD value of the cell solution used for measurements had a minor influence on the disintegration efficiency of sonication. Only for the stationary phase, the cell disruption effect was decreased with the increasing cell concentration. In the lag phase, the living cells in the solution, especially at 0.05 OD, were reduced to a greater extent after 360 s of exposure. In contrast, stationary-phase cells were more resistant to the ultrasonic treatment at all OD values.

The size distribution and electrokinetic properties of the un-sonicated *P. kessleri* cells, which were dispersed in distilled water, were determined in the lag, log, decline and stationary phases of growth. The results are presented in Fig. 4.

The size of *P. kessleri* cells (Fig. 4a) increased with the time of cultivation ($p < 0.05$). The effect of age of *P. kessleri* cells on the cell size decreased with an increasing OD_{650} value ($p < 0.05$ for $OD_{650} = 0.05$, $p = 0.05$ for $OD_{650} = 0.075$, $p > 0.05$ for $OD_{650} = 0.15$).

The mean hydrodynamic diameter of *P. kessleri* cells increased with the increasing optical density of the suspension ($p < 0.05$). Generally, there were no differences between values obtained for the 0.05 and 0.075 OD_{650} (i.e. about 25 and 42 million cells per millilitre, respectively). The mean hydrodynamic diameter was lower than 6000 nm for these samples. After the increase in the cell number to about 85

Fig. 3 Number of *P. kessleri* cells during sonication at different optical densities for **a** 24-h, **b** 96-h, **c** 240-h and **d** 360-h algal cells; the results are presented as the means of six measurements from $n = 3$; error bars

represent standard deviation; the blue lozenges, red squares and green triangles represent OD_{650} values of 0.05, 0.075 and 0.150, respectively

million per millilitre ($\text{OD}_{650} = 0.15$), the apparatus recorded large particles. The effect of OD_{650} on the determined size was the highest for cells in the lag and log phases ($p < 0.05$), and then it decreased ($p < 0.05$) to be statistically insignificant for the oldest cells ($p > 0.05$).

The values of electrophoretic mobility (Fig. 4b) of the *P. kessleri* cells dispersed in distilled water were negative. The electrophoretic mobility was not affected by the physiological stages of *P. kessleri* cells ($p > 0.05$) and the concentration of the cells in the suspension ($p > 0.05$).

The application of ultrasounds led to changes in the size of single algal cells, which can be seen in Fig. 5. The relative hydrodynamic diameter (Rd_t) was dependent on

the physiological stage of *P. kessleri* culture ($p < 0.05$). The highest values of Rd_t , which means the lowest reduction of size, were obtained for the logarithmic phase. The results recorded for these samples were characterised by high values of standard deviations. This was probably connected with the high heterogeneity of cells in the logarithmic phase of growth associated with active proliferation of the cells. The lowest values of Rd_t were obtained for the decline and stationary phases. However, it should be remembered that the size of the control cells (without the sonication) was the biggest in this case (Fig. 4a). Generally, the values of Rd_t depended on the optical density of the samples ($p < 0.05$).

Table 1 Summary of algal cells reduction (CR_{ns} (%)) for different OD values and different growth phases in *P. kessleri* culture

Time of sonication (s)	CR_{ns} (%)											
	$OD_{650} = 0.05$				$OD_{650} = 0.075$				$OD_{650} = 0.15$			
	Growth phase (h)				Growth phase (h)				Growth phase (h)			
	24	96	240	360	24	96	240	360	24	96	240	360
10	1.65	6.08	3.66	1.22	8.42	20.16	0.87	0.78	8.13	6.55	0.54	5.41
20	0.93	8.78	5.13	0.00	21.29	12.90	2.34	2.02	0.00	7.86	13.26	1.54
30	6.17	1.49	5.13	3.05	20.05	20.97	1.73	6.98	3.25	9.61	10.33	16.99
40	0.49	9.12	3.74	2.74	20.30	16.53	5.45	1.40	6.78	19.21	20.65	21.62
50	35.31	13.85	9.89	1.52	29.70	12.50	8.66	15.66	21.41	16.16	21.74	11.20
60	33.33	15.88	10.62	1.59	31.93	27.82	19.48	7.29	29.54	52.40	23.04	17.76
90	29.01	12.84	23.44	0.00	38.61	31.05	29.00	14.42	32.52	56.77	26.96	15.68
180	60.70	35.47	42.86	2.44	46.53	37.90	35.50	24.84	32.25	53.28	41.85	17.07
360	72.84	46.96	54.21	9.27	58.17	49.60	49.78	28.99	52.85	61.14	44.57	38.61

The relative hydrodynamic diameter (Rd_i) was strongly affected by the time of the ultrasound treatment ($p < 0.05$). Its values decreased with the prolongation of the sonication time. This was observed for all samples.

Additionally, there were statistically significant correlations ($p < 0.05$) between the relative hydrodynamic diameter (Rd_i) and the time of sonication (t) and between the relative hydrodynamic diameter (Rd_i) and the algal cell reduction (CR_{ns}). The relationships were used for calculation of the sonication time that is required for total disintegration of cells ($t_{100\%}$). The mathematical formulas, values of the correlation coefficient and values of $t_{100\%}$ are collected in Table 2. The time needed for the total disintegration of microalgal cells was longer than that applied in this study (410–3665 s). Generally, the effective time of sonication was dependent on the growth phase of *P. kessleri* cells. The longest $t_{100\%}$ was obtained for

stationary-phase cells in a suspension at $OD_{650} = 0.05$. However, the lowest values of correlation coefficients were determined for these samples (0.42–0.43).

The reduction of intact cells caused by ultrasounds was strongly correlated with the relative mean hydrodynamic diameter as well as electrophoretic mobility, mainly at the low concentrations of the cells. The relationship between the electrophoretic mobility and the CR_{ns} for varied OD_{650} values at the different physiological stages of *P. kessleri* cells are presented in Fig. 6. The electrophoretic mobility in the microalgal cell suspensions decreased (the absolute value) with the prolonged time of cultivation and ageing of the *P. kessleri* culture ($p < 0.05$) and with the increasing OD_{650} value ($p < 0.05$).

The prolonged exposure to ultrasound and the consequent decrease in intact cells in the solution led to an increase in the electrophoretic mobility ($p < 0.05$), which can be seen in Fig. 6.

Fig. 4 Values of the mean hydrodynamic diameter d (a) and electrophoretic mobility (b) (columns) as well as the number of cells (points) at the different growth phases; error bars represent standard deviation. Values of the mean hydrodynamic (d) and electrophoretic

mobility are presented as columns. Colours of the column: white, grey and dotted and colours of the dots: white, grey and black denote values of the parameters for OD 0.05, 0.075 and 0.15, respectively

Fig. 5 Relationship between the relative mean hydrodynamic diameter (Rd_t) and time of sonication for **a** 24-h, **b** 96-h, **c** 240-h and **d** 360-h algal cells in the suspension at different OD_{650} values; error bars represent

standard deviation; the white, grey and black colours represent OD_{650} values of 0.05, 0.075 and 0.150, respectively

Table 2 The values of sonication time which is needed for total rupture of the *P. kessleri* cells and the formulas, which were used for its calculation basing on Rd_t

Growth phase (h)	OD_{650}	$Rd_t = f(t)$		$Rd_t = f(CR_{ns})$		$t_{100\%}$ (s)
		Formula	r	Formula	r^c	
24	0.05	$y = 0.4612e^{-0.003x}$	0.68	$y = 0.4939e^{-0.013x}$	0.74	410
	0.075	$y = 0.3812e^{-0.004x}$	0.66	$y = 0.6588e^{-0.031x}$	0.91	638
	0.15	$y = 0.3609e^{-0.005x}$	0.76	$y = 0.4465e^{-0.033x}$	0.85	617
96	0.05	$y = 0.7244e^{-0.004x}$	0.86	$y = 0.8072e^{-0.028x}$	0.88	673
	0.075	$y = 0.6252e^{-0.003x}$	0.68	$y = 0.9264e^{-0.029x}$	0.77	835
	0.15	$y = 0.3440e^{-0.005x}$	0.76	$y = 0.4856e^{-0.028x}$	0.88	491
240	0.05	$y = 0.3865e^{-0.004x}$	0.71	$y = 0.4086e^{-0.023x}$	0.75	561
	0.075	$y = 0.3386e^{-0.004x}$	0.67	$y = 0.3793e^{-0.030x}$	0.78	721
	0.15	$y = 0.3498e^{-0.005x}$	0.76	$y = 0.5455e^{-0.041x}$	0.92	731
360	0.05	$y = 0.1972e^{-0.003x}$	0.43	$y = 0.1979e^{-0.110x}$	0.42	3665
	0.075	$y = 0.3279e^{-0.004x}$	0.70	$y = 0.3877e^{-0.052x}$	0.79	1258
	0.15	$y = 0.3123e^{-0.004x}$	0.61	$y = 0.4307e^{-0.046x}$	0.69	1070

Growth phase (h) = growth phase of cells expressed in hours, OD_{650} = optical density of cell suspension measured at 650 nm, $Rd_t = f(t)$ relationship between relative hydrodynamic diameter and the sonication time (s), $Rd_t = f(CR_{ns})$ relationship between relative hydrodynamic diameter and algal cell reduction (%), r correlation coefficient (at $p < 0.05$), $t_{100\%}$ the sonication time (s) needed for the total disruption of *P. kessleri* cells

Fig. 6 Relationship between the electrophoretic mobility and CR_{ns} for **a** 24-h, **b** 96-h, **c** 240-h and **d** 360-h algal cells at different OD_{650} values of the suspensions; the symbols: white circle, grey triangle and black

rhombus reflect the OD_{650} of 0.05, 0.075 and 0.150, respectively; (error bars represent standard deviation)

For the low optical densities (OD_{650} 0.05 and 0.075) of *P. kessleri* cells in the dispersion, there were statistically significant ($p < 0.05$) correlations between the values of electrophoretic mobility and the time of ultrasound exposure and between the electrophoretic mobility and CR_{ns} . The mathematic formulas, correlation coefficients (r) and the calculated values of time needed for the total disintegration of *P. kessleri* cells ($t_{100\%}$) are collected in Table 3.

The calculated time that was sufficient for the total disintegration of *P. kessleri* cells was in the range from 446 to 3634 s

and increased with ageing of the *P. kessleri* culture. This tendency was the same as that observed for $t_{100\%}$ which was calculated based on the Rd_t values. Although the cell disruption could take up to 60 min, the most intensive changes in the physical (size) and physicochemical (electrophoretic mobility) properties occurred within the first 60 s of sonication. In most cases, the prolongation of the time of the ultrasound action from 90 to 360 s did not cause significant changes in the diameter of the algal cells or the values of electrophoretic mobility (Figs. 5 and 6). Generally, as time progressed, the

Table 3 The values of sonication time which is needed for total destruction of the algae cells and the formulas, which were used for its calculation basing on electrophoretic mobility (U_e)

Growth phase (h)	OD_{650}	$U_e = f(t)^a$		$U_e = f(CR_{ns})^b$		$t_{100\%}^d$ (s)
		Formula	r^c	Formula	r^c	
24	0.05	$y = -0.0015x - 1.8157$	0.72	$y = -0.0071x - 1.7743$	0.81	446
96	0.05	$y = -0.0010x - 1.6881$	0.85	$y = -0.0070x - 1.6670$	0.81	679
	0.075	$y = -0.0014x - 1.5758$	0.94	$y = -0.0107x - 1.4470$	0.93	672
240	0.05	$y = -0.0016x - 1.5591$	0.94	$y = -0.0097x - 1.5415$	0.95	595
	0.075	$y = -0.0012x - 1.4526$	0.82	$y = -0.0079x - 1.4338$	0.85	643
360	0.05	$y = -0.0015x - 1.6291$	0.86	$y = -0.0544x - 1.6401$	0.76	3634

Growth phase (h) = growth phase of cells expressed in hours, OD_{650} = optical density of cell suspension measured at 650 nm, $U_e = f(t)$ relationship between electrophoretic mobility ($10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) and the sonication time (s), $U_e = f(CR_{ns})$ relationship between electrophoretic mobility ($10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) and algal cell reduction (%), r correlation coefficient (at $p < 0.05$), $t_{100\%}$ the sonication time (s) needed for the total destruction of cells

disruptive effect slowed down. The tendency of the cell diameter to decrease at the simultaneous increase in the electrophoretic mobility indicated that the disintegration still occurred. However, the efficiency of this process was low.

Discussion

The main determinants of effective disintegration of microalgal cells are the algal type, cell size and shape, cell wall strength and growth phase. Exposure to ultrasound treatment is proportional to damage to cellular biomass (Park et al. 2017). The resistance of stationary-phase cells of *P. kessleri* and the low reduction of the cellular population after 360 s of exposure generally result from differences in cell morphology (cell wall thickness, size and composition). In our previous report, ultrasonication was demonstrated as an appropriate technique in rupturing microalgal cells during extraction, and treating the sample with ultrasonic waves for 90 s improved lipid recovery from *Auxenochlorella protothecoides* cells (Piasecka et al. 2014). Joyce et al. (2014) reported that no intact cells of *Dunaliella salina* remained after a 4-min sonication treatment, but in the case of *Chlamydomonas concordia* cells, 16-min sonication ruptured cells completely. *Chlorococcum* cells remained intact even after continuous sonication at the maximum power level (130 W) for 25 min (Halim et al. 2012). Differences among examined green algal species are a direct consequence of cell wall thickness and structure. Generally, species with a thinner cell wall were easier to disrupt (Halim et al. 2013). *Parachlorella kessleri* cells are characterised by a thicker (54–59 nm) cell wall than that in other '*Chlorella*' species (Yamamoto et al. 2005). Juárez and Vélez (2011) reported that the thickness of the cell wall could reach up to 60 to 80 nm. Additionally, the fibrillar cell wall component of *P. kessleri* cells was composed of 100% of *N*-acetylglucosamine (chitin) (Takeda 1991; Juárez and Vélez 2011).

In the un-sonicated *P. kessleri* samples, the increase in the cell size with the time of cultivation was a result of the growth process, during which the volume of a particular cell and, in consequence, its diameter became bigger. According to Chioccioli et al. (2014), during the growth cycle, the cell size is changing predictably. In some tested species, there was a strong correlation between the size and shape of cells and environmental conditions. Chioccioli et al. (2014) reported that *Chlorella vulgaris* stationary-phase cells began to increase in size. This tendency was observed in this study, especially at the low concentration of algal cell suspension.

The OD₆₅₀ affected the measured size of the algal cells due to the restricted diffusion in concentrated samples. The DLS method is based on the analysis of light scattered by particles that are moving due to Brownian motion. The diffusion coefficient of particles is inversely proportional to their size (Eq. (5).

In the concentrated system, the diffusion of particles was inhibited. Considering the above, the value of the hydrodynamic diameter obtained by DLS was higher in respect to its real value (Pecora 1985; Zetasizer Nano Series User Manual, 2004; ISO 22412:2017). For the *P. kessleri* cells in the stationary phase, the effect was masked because the cells were large and their diffusion was slow. To avoid this problem in the analysis of the influence of the sonication treatment on the cell size, the relative values of the mean hydrodynamic diameter (Rd_i) were used instead of the apparent ones.

The negative values of electrophoretic mobility of the *P. kessleri* cells dispersed in distilled water suggest that the external cell envelope is dominated by acidic functional groups, the dissociation of which generates a negative electrical charge of the surface. At the neutral pH (about 7), there are mainly carboxylic (-COOH) and phosphoryl (-POH) groups (Vandamme et al. 2013; Ndikubwimana et al. 2015).

The effective time of sonication, determined on the basis of the relationships between the relative hydrodynamic diameter (Rd_i) and the time of sonication (t) as well as between the relative hydrodynamic diameter (Rd_i) and the algal cell reduction (CR_{ns}), was dependent on the growth phase of *P. kessleri* cells. The cell walls of the cells from the stationary phase were more resistant to the ultrasound treatment than the cell walls from the preceding physiological stages. This may be a consequence of different thickness of cell walls. Yamamoto et al. (2005) reported that *P. kessleri* cells from the late growth phase were characterised by a thicker cell wall than that in early growth phase cells.

The mobility of particles in liquid, which is caused by the applied electric field, is higher for small particles possessing a surface electric charge than for big ones (Hunter 1988). Therefore, the disintegration of *P. kessleri* cells should result in an increase in the absolute value of electrophoretic mobility due to the appearance of small particles in the system. In fact, the fragmented debris appeared among the *P. kessleri* cells in the cell suspension due to the sonication process. The reduction of intact cells caused by ultrasounds was strongly correlated with the relative mean hydrodynamic diameter as well as electrophoretic mobility, what has not been shown so far. The stationary-phase cells were bigger than the young ones, as discussed earlier, and this resulted in their slower movement. Additionally, the increase in the suspension density caused blocking of the movement by the neighbouring particles, which led to a decrease in the electrophoretic mobility. The action of ultrasounds resulted in an increase in the electrophoretic mobility values. This was caused probably by the release of cellular content to the bulk solution and the high mobility of disintegrated cells as well as dissociation of functional groups, which may be facilitated by the applied ultrasounds.

The analysis of both the cell size and electrophoretic mobility in the sonicated samples allowed estimation of the time of ultrasound action required for efficient and complete algal cell

disintegration. This points out the usefulness of the applied laser techniques in the algae studies.

Conclusions

Based on the results, it can be concluded that the laser light scattering methods can be applied in investigations of algal cell wall disruption. The effect of ultrasonic treatment on disruption of *P. kessleri* cells was determined from the physical and physicochemical properties of algal cells by laser light scattering methods and the algal cell reduction CR_{ns} . These results indicate electrophoretic mobility as a new parameter to investigate the effectiveness of cell disruption prior to extraction in algal bioproduction processes. Additionally, these studies enable us to estimate the time required for efficient and complete algal cell lysis.

Funding This work was funded in part by the National Science Centre, Poland (Grant No. 2017/25/N/NZ9/01785) in 2018–2021.

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